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1991 RESEARCH SUMMARIES

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1925 Linden Drive West
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Agricultural Research Service

United States Department of Agriculture

March 1992

U.S. DAIRY FORAGE RESEARCH CENTER, USDA-ARS
Madison, WI 53706

Dear Reader:

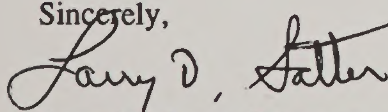
It is a pleasure to update our progress by bringing you these summaries of recent research. The U.S. Dairy Forage Research Center is a unique part of the national research program of the Agricultural Research Service, U.S. Department of Agriculture. The Center's mission is to build a knowledge and technology base for the dairy industry to fully exploit the use of forages in the production of milk. The Center has agricultural engineers, plant and soil scientists, microbiologists, ruminant nutritionists and a chemist working together to increase the efficiency of forage production and utilization by dairy farmers. We function in close cooperation with the Agricultural Experiment Stations of several states. The Center is located on the campus of the University of Wisconsin, Madison, and has "Cluster" locations in St. Paul, MN, Ames, IA, East Lansing, MI, and Ithaca, NY. The Center's research farm with facilities for 300 milking cows is located on 63 acres of USDA land on the banks of the Wisconsin River in Prairie du Sac, WI. An additional 1200 acres of adjacent land is utilized by the Center by agreement with the U.S. Department of the Army. The Center was established in 1980 and has made steady growth since. At present there are fifteen scientists: ten at Madison, and one at each of three Cluster locations, and two at the St. Paul, Minnesota Cluster location. Scientists hold faculty appointments in university departments and provide supervision for approximately 15-20 graduate students and 5-10 post doctoral fellows.

The U.S. Dairy Forage Research Center sponsored the International Symposium on Forage Cell Wall Structure and Digestibility in October. It was a great success and we thank the Cell Wall Work Group (Buxton, Hatfield, Jung, Mertens, Ralph and Weimer) for planning and hosting the symposium. This group has been exceptionally successful in developing multidisciplinary research approaches to forage cell wall problems. We are planning to enhance the activities of two other work groups within the U.S. Dairy Forage Research Center. The "Systems" work group will be strengthened by the addition of a Plant Scientist who will be developing computer models of crop growth, integrating these with other components of DAFOSYM, a dairy forage system model developed by Dr. Rotz. A protein work group has just been established under the leadership of Dr. Broderick to coordinate and develop research approaches for improving utilization of forage protein by dairy cows. Much of the protein in grass and legume forages is readily degraded by microbes in the first compartment of the ruminant stomach. Improving utilization of forage protein could dramatically lower the cow's need for supplemental protein, thus reducing the cost of milk production.

We have increased our investment in equipment and physical plant at our research farm to improve efficiency, reduce costs and increase our capability for doing research. Our new forage wagon enables us to load trucks for hauling chopped forage from the field, thus speeding up the forage harvest. We made some major improvements in the milking parlor and in one of our free stall barns, built an additional bunker silo and constructed a chemical storage/handling facility. We are initiating projects to improve ventilation in the two tie stall barns and to install energy efficient lighting throughout our facility. We are also in the midst of a review of all our management practices at the research farm, again hoping to improve our effectiveness in carrying out research.

We are pleased and very proud of the way Center scientists from diverse disciplines interact and bring their collective insights to bear on the problems of forage production and utilization. This collection of research summaries illustrates the progress they are making in developing information to help dairy farmers utilize their resources more effectively. The research is intended to benefit producers of forage crops, dairy farmers and the consumers of dairy products.

Sincerely,



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Acknowledgment

Appreciation is expressed to Gloria Palmer for her interest in and dedication to the task of typing and assembling this annual research summary.

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PUBLICATIONS

DAFOSYM: THE DAIRY FORAGE SYSTEM MODEL

C.A. ROTZ and P.W. WILKENS

Introduction

For the past 15 years a major effort has been given to the development of a simulation model of the dairy forage system called DAFOSYM. The model simulates the growth, harvest, storage and utilization of alfalfa and corn crops on the dairy farm. DAFOSYM was originally developed as a research tool for evaluating alternative technologies and management strategies for forage production and utilization on the dairy farm. Many analyses of alternatives in forage harvest and storage have been performed with the model. As the model developed, it became applicable for more general conditions and it became easier to use by those not involved in the model development. This development has led to the desire for an even more user-friendly model for use as a teaching aid in classroom and extension teaching programs.

Materials and Methods

To create a more user friendly model, a new user interface was developed. The new interface uses overlaying menus to lead the user through data preparation and execution of the model. All data entries are selected from listed options or they are entered as real numbers. No code numbers are used for soils, machines, silos, etc. for easier selection. The new menus provide greater ease and more rapid use of the model by inexperienced users. A help key is also provided to supply extended help messages on each data entry. A mouse can be used for greater ease and speed in manipulating menu information.

To provide greater versatility for DAFOSYM, several programs were prepared for manipulating input and output parameter files. Programs were written for viewing, editing and printing input parameter files required for a simulation. Another program was created for viewing and plotting output data.

Other improvements made to DAFOSYM during the past year include new routines for alfalfa harvest, hay drying and hay preservation. The harvest routine was rewritten to provide a more logical and modular approach to harvest simulation. The new routine simulates harvest through five periods of the day. This approach allows the model to be more versatile for future development. The hay storage subroutines were expanded to model chemical preservation and barn drying of hay baled at higher than normal moisture levels.

Results and Discussion

The new DAFOSYM (version 4.0) includes a package of programs linked through a master menu to aid the user in preparing input files and reviewing output files. The first of these programs called PLOTTER is used to plot output information from DAFOSYM for better verification of simulation results. A variety of graphs, bar charts, pie charts and cumulative probability distributions can be created. Plots include such things as daily alfalfa yield and quality during growth, harvested yield and quality by cutting, breakdowns of production and feeding costs and probability distributions of annual yields, costs and net returns for representative farms.

Programs are also provided for creating, editing and printing the various input files. These programs are FARMGEN, MACHGEN, CORNGEN and WTHRGEN. FARMGEN creates and edits farm data files used to describe the representative farm being modeled. MACHGEN uses a spreadsheet type of approach to view and edit parameters for machinery and silos available for use by DAFOSYM. CORNGEN is used to create, view and edit the corn data file

used by DAFOSYM. CORNGEN includes a simulation model called CERES-maize which generates corn growth information for specific locations, soil types, corn varieties, etc. WTHRGEN creates weather data used by DAFOSYM and CORNGEN for specific locations when real data are not available. A model called WGEN is used in WTHRGEN to create typical weather patterns. WTHRGEN also includes the ability to view, statistically analyze and plot weather data files.

A user's guide was prepared which demonstrates the use of the DAFOSYM package of programs. During the next year the model and user guide will be distributed to interested users.

Conclusion

DAFOSYM version 4.0 provides a very friendly and versatile software package for use as a teaching aid on dairy forage systems. The model provides an excellent tool for demonstrating the many components of forage production and utilization and the interrelationship of these components. After attaining experience with the model, a user may also use it as an aid in making strategic plans for a given farm.

FORAGE ALFALFA AND CROP SEQUENCING MODELS

P.W. WILKENS, C.A. ROTZ and J.T. RITCHIE

Introduction

Modelling crop growth to maximize production and minimize environmental impact requires a thorough understanding of the soil-plant-atmosphere continuum. Progress continued in the development and calibration of the dynamic alfalfa simulation model, Forage ALFALFA, and a new crop sequencing simulation model, SALUS. SALUS (Sustainable Agricultural Land Use System) is a crop sequencing model that links crop growth simulation models of the IBSNAT-DSSAT system on a common soil model. Long term crop sequencing simulations including forages can be used to test alternative management strategies on the sustainability of agricultural cropping systems. Environmental assessment of real world cropping systems is not easily accomplished using single, monoculture simulation systems. Rotations may include cereals, grain legumes, forage legumes, and/or fallow (Table 1). In addition, each crop model may be run as a stand alone component model.

Methods and Materials

A number of substantive changes were incorporated into the SALUS and ALFALFA models during the past year, including:

1. A new soil water balance. A one dimensional soil profile with predetermined layer thickness was utilized. A static or fluctuating water table was used with drainage and redistribution of water in the profile. Ponding and runoff were based on rainfall intensity and hydraulic conductivity with simulated rainstorms. Root water uptake and water deficit stresses are calculated.

2. An integrated weather generation model (WGEN). The weather generator allows simulated weather for any location to be generated during a cropping sequence simulation. Controls allowing analysis of climate modification (CO_2, temperature, and rainfall) were also added.

3. An improved root growth routine. In the new model, growth of roots is through axes and branches and the growth is affected by soil physical factors (soil strength, temperature, nutritional status, and aeration

supply). Partitioning of carbohydrate between axes and branches is controlled by a genetic parameter influencing the range of root growth between strong tap-rooted and fibrous growth types.

4. An improved soil nutrient routine. Initial versions of subroutines involving manure fertilization (nitrification, denitrification, and ammonia volatilization), phosphorus uptake and soil dynamics, and tillage were incorporated.

A draft version of a user's manual for Forage ALFALFA was completed for distribution with the model in 1992. Data input files will be converted to the DSSAT Version 3.0 standard upon the publication of the file format specifications in February 1992.

Results and Discussion

Validation of the Forage ALFALFA model indicates that it effectively simulates growth, N-fixation, and phenological development under non-stress conditions. Further work is required on the timing of phenological events and the dynamic changes in herbage quality under stress conditions. Additional genetic parameters may be required to describe differential responses between cultivars to the environment. Experimental research is continuing on alfalfa response and recovery from deficit water conditions. Functional relationships derived from field research and published data are incorporated into the model to improve model performance under deficit conditions.

Forage ALFALFA is a component model of SALUS, and as such, cropping systems with alfalfa (including management strategy, cropping sequence, irrigation, and fertilization) can be evaluated for any combination of soil type and climate for extended periods. Optimization for both environmental quality and yield stability can be accomplished for any designated cropping system, including a comparison of different forage crops within a dairy system.

Conclusion

Forage ALFALFA and the other crop models used with SALUS provide an excellent research tool for studying nutrient management and the environmental impacts which occur under various cropping sequences. A user's manual for the SALUS model and release Version 1.0 will be ready for distribution in 1992.

Table 1. IBSNAT crop growth models included in SALUS Vers. 1.0.

Crop	Model	Species	Developer
Maize	CERES-Maize	Zea mays	Ritchie et al.
Wheat	CERES-Wheat	Triticum vulgare	Godwin et al.
Soybean	SOYNIT	Glycine max	Jones et al.
Potato	SUBSTOR	Solanum tuberosum	Griffin et al.
Alfalfa	Forage ALFALFA	Medicago sativa	Wilkens et al.
Barley	CERES-Barley	Hordeum vulgare	Otter-Nacke et al.
Millet	CERES-Millet	Pennisetum glaucum	Alagarswamy et al.
Sorghum	CERES-Sorghum	Sorghum bicolor	Ritchie et al.
Dry Bean	BEANGRO	Phaseolus vulgaris	Hoogenboom et al.

PERFORMANCE OF MORPHOLOGICALLY DIVERSE ORCHARDGRASS CLONES IN SPACED AND SOLID PLANTINGS

D.R. BUXTON and E.M. LENTZ

Introduction

During early stages of a breeding program, most perennial forages are evaluated under spaced planting conditions for ease of identification of individual plants. Forage production, however, occurs under solid-seeded, sward conditions. Limited information is available regarding the response of perennial forage genotypes to plant density. We previously reported that orchardgrass plants grown in spaced-planted conditions and selected for wide leaf blades often were more digestible during spring growth than those selected for narrow blades. This correlated response is of value for early screening of orchardgrass germplasm. The current study was conducted to determine if similar relations hold when plants are grown under solid-seeded conditions and if observations made in spaced plantings can be used to make inferences about relative differences among genotypes grown in solid plantings.

Materials and Methods

Ten orchardgrass clones from two maturity groups (inflorescence emergence differed by 8 d) were selected for wide, narrow, long, or short blades. Additionally, a clone of a check cultivar was selected for each maturity group. The check cultivars were Napier (early maturity) and Orion (late maturity). Propagules of each clone were transplanted at two plant densities into the field. In the spaced planting, clones were located on 0.60-m centers and in the solid planting, clones were planted in a 4 x 4 grid spaced on 0.15-m centers. The experiment contained three replicates. Spring growth was harvested near June 1 when 50% of the late-maturity group had one or more inflorescences at anthesis. Regrowth herbage was harvested after 5 wk of forage accumulation. Samples were taken for plant measurements and in vitro digestibility determination by cutting plants at an 8-cm height. Reproductive tiller components were separated by removing blades at the collar, leaving sheaths and inflorescences with stems. Vegetative tiller components were separated into blades and sheaths.

Results and Discussion

There were few significant plant density interactions for the traits measured. Additionally, correlation coefficients relating performance of the clones grown in solid and spaced plantings were generally high (Table 1). Genotypes selected for wide blades were more digestible, especially in stems, than those selected for narrow blades in the spring (Table 1), with little difference during the summer. Plants from the early-maturity group were less digestible than those from the late group. In the spring, the difference in digestibility among maturity groups was 4% for reproductive tiller blades (625 vs. 650 g kg^{-1}), 23% for reproductive tiller stem + sheaths (535 vs. 659 g kg^{-1}), 2% for vegetative tiller blades (639 vs. 649 g kg^{-1}), 4% for vegetative tiller sheaths (673 vs. 699 g kg^{-1}), and 13% for total herbage (575 vs. 647 g kg^{-1}). In summer, the difference in digestibility between the maturity groups was small.

Conclusion

Our work indicates that plant breeders can make initial screenings of orchardgrass germplasm for wide-leaf blades under spaced-planted conditions and expect that the trait will be manifested in solid-planted yield tests. Furthermore, progress toward selecting orchardgrass with higher digestibility can be made by selecting genotypes with wide leaves. Our data suggest that selections will be most effective in spring-grown reproductive tillers but that good results may also be expected by making selections in summer-grown vegetative tillers because differences in blade width were also evident in the summer.

Table 1. *In vitro* digestion dry matter (g kg^{-1}) in plant parts and total herbage of orchardgrass harvested in the spring from solid or spaced plantings. Data are average of three replicates and two maturity groups.

Blade trait/	Reproductive tiller blades			Reproductive stem + sheaths			Vegetative tiller blades			Vegetative tiller sheaths			Total herbage		
source of variation	Solid	Spaced	Avg.	Solid	Spaced	Avg.	Solid	Spaced	Avg.	Solid	Spaced	Avg.	Solid	Spaced	Avg.
							In vitro digestible dry matter (g kg^{-1})								
Long	643	663	653	628	666	647	629	649	639	689	716	701	613	647	630
Short	608	640	626	534	638	592	650	671	661	687	717	702	584	642	613
Wide	628	667	653	595	647	625	635	668	654	665	735	705	632	668	653
Narrow	620	627	623	513	540	527	640	637	639	649	680	665	553	594	574
Check	630	644	637	606	619	612	625	644	634	645	693	670	603	632	617
Mean	627	648		576	620		636	653		667	707		593	634	
							Mean squares								
Plant density		3726**			27364**			2147*			17501**			20593**	
Maturity group		6037**			127655**			11			2756*			38496**	
Blade trait		1599**			24032**			2125**			4902**			7076**	
Maturity x trait		6979**			21589**			7350**			7351**			9409**	
Density x maturity		13			47			817			507			1160	
Density x trait		169			1309			341			257			397	
Density x maturity x trait		613			259			237			152			527	
CV (Error b)		3.0			5.1			2.4			3.2			2.9	
r†		0.88**			0.95**			0.85**			0.97**			0.87**	

*,**Significant at the 0.05 and 0.01 probability levels, respectively.
†Correlation coefficients for entries in solid vs. spaced plantings, n = 9.

GROWTH OF C_3 AHD C_4 PERENNIAL GRASSES IN REDUCED IRRADIANCE

K.D. KEPHART, D.R. BUXTON and S.E. TAYLOR

Introduction

Little comparative information exists on growth responses of C_3 (cool-season species) and C_4 (warm-season species) species to reduced irradiance. Morphological adaptation of leaves and stems to irradiance regimes should occur in both C_3 and C_4 species to irradiance level. Studying morphological adaptation to low light will add to our understanding of grass growth in dense canopies, in regions with prolonged cloudy weather, and in agroforestry systems. Adaptive changes in plant morphology to reduced irradiance likely will affect quality of the forage. The objective of this investigation was to determine morphological and growth responses of C_3 and C_4 forage grasses to reduced irradiance.

Methods

A field study was conducted using five grass species of differing photosynthetic type (C_3 vs. C_4), grown under three levels of irradiance. The C_3 species were tall fescue, reed canarygrass, and deertongue grass, and the C_4 species were switchgrass and big bluestem. Polypropylene fabric shades were used to impose irradiance treatments of 37 and 70% of available irradiance in comparison with a nonshaded control. Morphology and growth measurements were made at 21-d intervals beginning on 5 June in Year 1 and 28 May in Year 2. Net leaf carbon exchange rate (photosynthesis) of recently expanded leaves was measured for reed canarygrass and switchgrass 5 to 7 d before morphological and growth measurements. These measurements were made only during cloud-free periods near mid day.

Results and Discussion

Response of herbage yield, shoot dry-weight, and crop growth rate to irradiance was twice as great for C_4 grasses as for C_3 grasses (Figure 1). Although shoot length

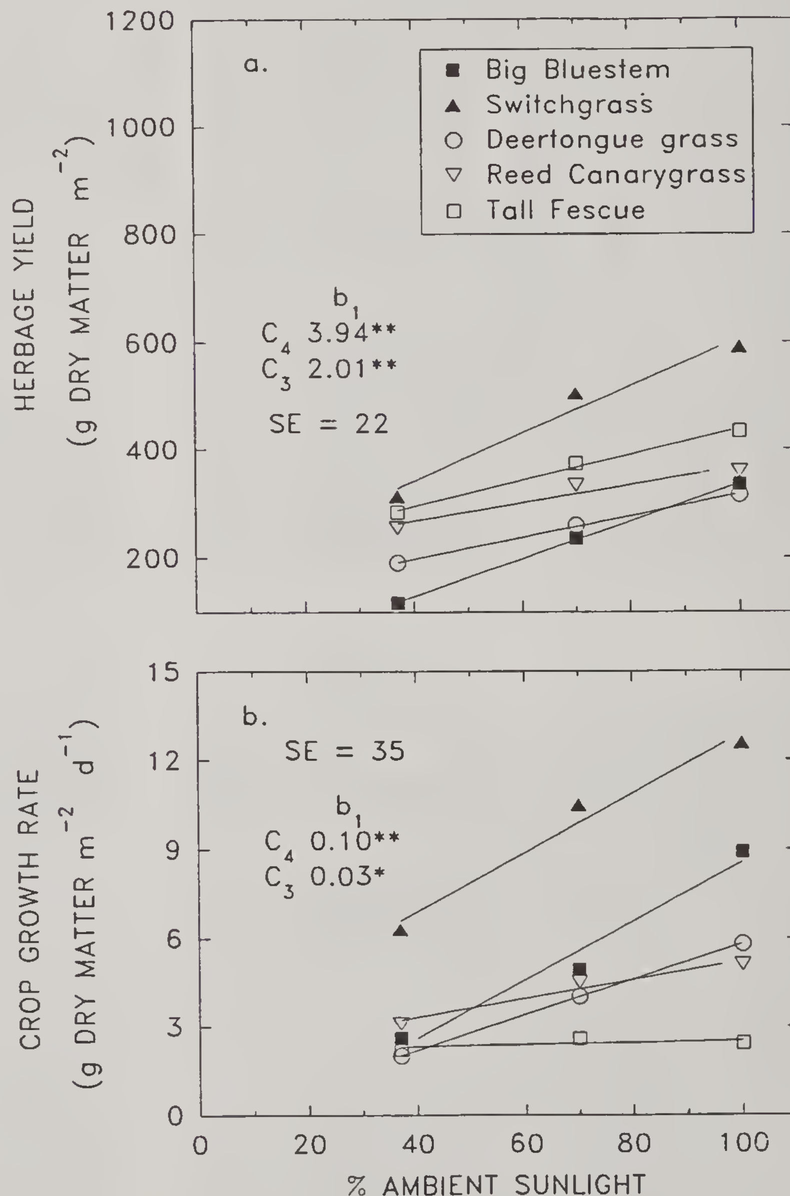


Figure 1. Linear regression (b_1) of herbage yield and mean crop growth rate to irradiance level for three C_3 and two C_4 grasses.

was not affected by irradiance, stem dry weight decreased with shade. Response of carbon exchange rate to irradiance reduction was greater for switchgrass than for reed canarygrass. The most consistent morphological response to increased sunlight involved changes of leaf blades. Specific leaf weight increased (thus, leaves were thicker or more dense in full sunlight than when grown in shade) and leaf-area ratio (leaf area per unit plant weight) decreased with increasing irradiance in a similar manner for all five species. Leaf-to-stem ratio did not respond consistently to reduced irradiance. Thus, morphological adaptive response was related to maintaining leaf area at the expense of leaf thickness or density. In early July of Year 2, photosynthesis was measured 24 h after shade fabrics were removed; 55 d after shading began. Adaptation to shade did not seem to affect photosynthesis in full sunlight. Leaves grown in 37 and 70% shade had photosynthetic rates comparable to those grown in full sunlight.

Conclusion

Morphological adaptation to environmental conditions may influence forage quality, utilization, and production of pasture grasses. Unlike effects of adaptation to drought and high temperatures, adaptation to shade had little affect on shoot length, maturity, or leaf-to-stem ratio, three important morphological traits that influence forage quality of total herbage. Shade reduced forage production more in C_4 grasses than in C_3 grasses even though morphological responses to shade seemed to be similar. Producers wishing to grow forage grasses under shaded conditions, such as in agroforestry, should expect forage yield to be reduced especially for C_4 species.

DIFFERENCES IN RESPONSE TO AVAILABLE PHOSPHORUS AMONG WHITE CLOVER CULTIVARS

C.J.P. GOURLEY, D.L. ALLAN and M.P. RUSSELLE

White clover (*Trifolium repens* L.) is an economically important forage species in temperate and sub-temperate environments. However, commonly used cultivars generally are not suited to soils with limited phosphorus (P) availability and usually require P fertilization for high yields. To reduce both production costs and the potential for surface water contamination by P in runoff from agricultural fields, there has been increasing interest in selecting plants that are more efficient in P uptake.

Because soil is a highly variable and complex medium, initial plant selection for low P tolerance is accomplished better in artificial cultures. The sand-alumina culture technique can provide solution P concentrations similar to those in soil and allows easy separation of roots from the medium for investigation of P uptake mechanisms. The objective of this research was to compare white clover cultivar response to a range of P concentrations in sand-alumina.

Materials and Methods

In the first experiment, six cultivars of white clover, purportedly P efficient (Gandalf), moderately P efficient (Huia and Clarence), and inefficient (Pronitro, El Lucero, and Haifa) were used, but two (Clarence and Haifa) were grown under low and high solution P concentrations only. Sand-alumina cultures were prepared by preloading the alumina with P, mixing the sand (2950 g) with alumina (50 g), and leaching with nutrient solution to remove excess P. After steady-state P concentrations of 0 to 88 μM were achieved, 16 pregerminated seeds of a given cultivar were transferred to each pot. Seedlings were

inoculated with appropriate *Rhizobium* 8 days after sowing. Plants were grown in the greenhouse with supplementary lighting and additions of nutrient solution (without P) at least every 3 days. Each cultivar and P concentration was replicated three times in a randomized complete block design. Plants were harvested 52 days after sowing and were separated into shoots, fine roots, and coarse roots. Organ dry weights were measured, P concentrations were determined, and P accumulation was calculated.

In the second experiment, Gandalf, Huia, and Pronitro were grown in sand-alumina at 5.7 or 82 μM solution P concentration for 33 days in a growth chamber. At harvest, roots were carefully rinsed free of the medium. Root tip numbers, average root diameter, root length, root hair density and average length, nodule number, and nodule effectiveness (visually rated by color) were determined.

Data were analyzed by standard analysis of variance. Several nonlinear plateau models were tested to describe the relationship between solution P concentration and both shoot dry mass and P accumulation. The model with the consistently highest R^2 values and best-fitting residual plots was the Michaelis-Menton equation:

$$y = (aP_s) / (b + P_s)$$

where y is the measured response variable (e.g., shoot dry mass) and where a and b were estimates of maximum dry matter or P uptake at one half maximum y.

Results and Discussion

The sand-alumina culture media proved effective for determining differences in white clover cultivar response to P (Fig. 1). Gandalf produced the greatest shoot dry mass and accumulated the most P at all solution P concentrations. Huia and Pronitro had similar responses for both characteristics. El Lucero accumulated an intermediate amount of shoot dry matter and P.

However, these cultivars did not fall in the same rank order for shoot mass at low P as at high P availability. For example, Clarence and Haifa shoot dry matter ranked 3 and 5 at 2.9 μM P but ranked 6 and 2 at 40 μM P. This high ranking for Haifa contrasts with other reports and stresses the importance of verifying in the field responses found under controlled conditions. Cultivars generally showed similar ranking for root characteristics and P accumulation as they did for shoot mass across all solution P concentrations (data not shown). This supports the premise that nutrient uptake per plant is closely related to shoot and root size.

Despite highly repeatable solution P concentrations among pots treated the same, the coefficient of variation for shoot dry matter yield was 19% and for P uptake was 22%. We suggest that most of this variation was due to differences among individuals within cultivars of this cross pollinated species.

None of the putative mechanisms of improved P efficiency we measured were consistently related to cultivar performance in this experiment. For example, some researchers argue that high tissue P concen-

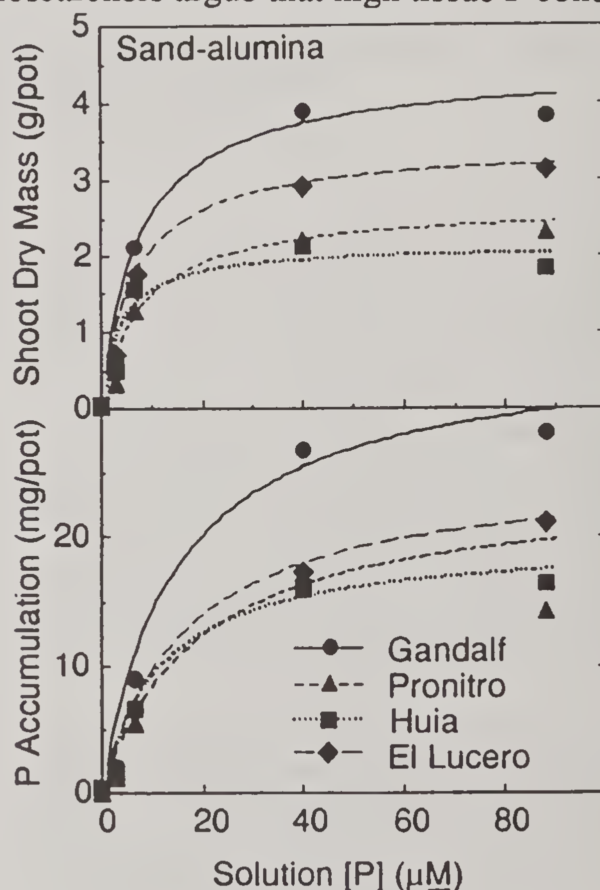


Figure 1. Relationship between solution P concentration in sand-alumina culture and shoot yield and P accululation of white clover cultivars.

tration may indicate tolerance to low P availability; others have argued the inverse, that low tissue P concentration may be related to more efficient metabolism of the element. We found no relationship between tissue P concentration and shoot dry mass, which calls into question both hypotheses. In addition, we found no differences in average specific P accumulation (g P/m root length), root tip number, root diameter, or root hair density and length (data not shown). Gandalf and Huia had greater nodule effectiveness than Pronitro under low P conditions in one experiment, but there were no differences in the other. In neither experiment did we find differences in nodule number among cultivars.

Conclusion

Results of this research have shown that marked diversity in shoot dry mass response to solution P concentration exists among white clover cultivars. Greater shoot yields and P accumulation were associated with larger root systems which presumably enabled greater P absorption. There was no evidence to suggest that P was absorbed by plant roots or utilized in plant tissues in a more efficient manner by the higher yielding cultivars. Therefore, dissimilarity in yield response to solution P availability cannot be discussed unambiguously in terms of P efficiency, as yield differences may be due to other factors.

NITROGEN DYNAMICS IN ALFALFA (*Medicago sativa* L.) AND BIRDSFOOT TREFOIL (*Lotus corniculatus* L.) ROOTS AND NODULES

M. DUBACH and M. P. RUSSELLE

This work was partially sponsored by the Swiss National Funds.

Introduction

Legumes can symbiotically fix atmospheric nitrogen. Some of this fixed N is transferred to non-legumes if legumes and non-legumes are intercropped. We do not know how this N transfer occurs. Our hypothesis is that significant amounts of N are potentially available for release into soil upon death of roots and nodules. As the first step in this research we monitored N concentration and N content of fine roots and nodules over their lifetime. We compared alfalfa with birdsfoot trefoil because their nodule physiology differs, even though these perennial legumes fix and transfer about the same amount of nitrogen.

Material and Methods

Ten alfalfa plants and ten birdsfoot trefoil plants were grown in root observation boxes in a growth chamber for 4 months. Root growth was recorded at 3- to 7- day intervals. Herbaceous plant parts were harvested two or three times. The final harvest took place either 2 or 5 weeks after the previous harvest. On the day of final harvest we removed the living fine roots and nodules according to their age. The term fine roots includes all roots that did not show any secondary thickening. We also sampled the rhizosphere and nodusphere soil, which is the soil surrounding the roots and nodules, respectively. All samples were analyzed for total N (Dumas combustion) and total C. We corrected the N concentration of root and nodule samples for contamination by soil by assuming that any decrease in sample C concentration from 450 mg C g^{-1} was due to soil with C and N content like the rhizosphere sample.

Results and Discussion

The C and N contents of alfalfa and trefoil roots younger than 1 week are shown in

Table 1. Plants were harvested 14 days after the preceding herbage removal.

Fine roots of alfalfa had a significantly higher N concentration than trefoil roots. Their specific root weight was also greater. Specific N content (mg N cm^{-1}) was therefore almost twice as high for fine alfalfa roots compared with trefoil roots (2.4 vs. 1.3 mg N cm^{-1}).

N concentration of roots decreased over the first 2 to 4 weeks and was stable thereafter at a relatively high level of about 35 mg N g^{-1} for alfalfa and 30 mg N g^{-1} for trefoil over the observed lifespan of the roots of 10 to 12 weeks (data not shown). The C:N ratio increased during this time. There was no significant difference between the specific root weight and the specific N content over time. This indicates that a certain length of fine roots contained the same amount of N, no matter how old the roots were. This was true for both alfalfa and birdsfoot trefoil fine roots.

Active birdsfoot trefoil nodules had an N concentration of over 86 mg g^{-1} and the specific nodule weight was about half a milligram. Inactive (green) trefoil nodules showed the same N concentration and N content as large, living alfalfa nodules. The interesting point here is that almost 50% of the N originally present in active trefoil nodules disappeared when they became inactive. Because there was no significant increase in N concentration of nodusphere soil, we assume that this N was reabsorbed by the trefoil plants. We do not have similar results for alfalfa because we were not able to distinguish between inactive and dead nodules. The difference between living alfalfa and birdsfoot trefoil nodules is due to their different nodule physiology. Alfalfa nodules have an indeterminate meristem. They are able to live for an extended period of time and therefore old and young tissue are present in a nodule. Trefoil nodules have a determinate meristem; they only live for a limited amount of time and the nodule is more homogeneous.

Conclusions

Fine roots of alfalfa had on the average a 55% higher N content per unit root length than fine birdsfoot trefoil roots, but the N content cm^{-1} of fine roots of both alfalfa and trefoil did not change over time. The lack of a decrease in specific N content in older fine roots of alfalfa and trefoil indicates that there was no significant N remobilization, or that N import equalled N export in these tissues. Large active trefoil nodules (> 3 mm) had twice the N content of active alfalfa nodules, but when trefoil nodules turned inactive, 50% of their N content apparently was reabsorbed by the plant.

These results suggest that there is a significant amount of N potentially available for release into the soil upon death of roots and nodules.

Table 1. Comparison between fine roots of young alfalfa and birdsfoot trefoil (< 7 days old; n=4). Standard deviations are in parentheses.

Species	N concentration [mg N g^{-1}]	N content [µg N cm^{-1}]	Specific root mass [µg cm^{-1}]	C:N ratio
Alfalfa	46.0 (2.5)	2.4 (0.3)	54 (9)	9.9 (0.5)
Trefoil	38.5 (1.8)	1.3 (0.1)	35 (1)	11.8 (0.6)

Table 2. Comparison between alfalfa and birdsfoot trefoil (n=4).

	N concentration [mg N g^{-1}]	N content [µg N nod^{-1}]	Specific nodule mass [µg nod^{-1}]	C:N ratio
active alfalfa nod. >3 mm	66.9 (1.1)	24.3 (5.2)	360 (74)	6.7 (0.1)
active trefoil nod. >3 mm	86.6 (0.9)	45.6 (3.5)	526 (39)	5.2 (0.1)
inactive trefoil nod. >3 mm	65.9 (2.5)	23.8 (3.6)	360 (53)	6.9 (0.3)

SELF-INCOMPATIBILITY IN SEXUALLY-DERIVED TETRAPLOID RED CLOVER

V. MEGLIC and R.R. SMITH

Introduction

In the natural form, red clover is a highly self-incompatible, cross pollinated diploid (2n=2x=14) forage species. There are no naturally occurring polyploid forms, although chemically- and sexually-derived tetraploids (2n=4x=28) exist. Some tetraploids, especially those induced using chemical agents, exhibit a degree of self-compatibility which in turn introduces a certain level of inbreeding. This study describes the self seed production of nine tetraploid populations of red clover derived either sexually (unilateral or bilateral) or chemically (nitrous oxide or colchicine).

Materials and Methods

Five chemically- and four sexually-derived tetraploid red clover populations were examined in this study. Three populations (C21, Temara and Sally) were derived using colchicine, two (Kenstar 4x and Vanessa) were derived using nitrous oxide, two (Bi4x F_2 and Bi4x F_4) were derived by bilateral sexual hybridization (2x-2x crosses), and two (TW2x4 and Ken 2x4) were derived by unilateral sexual hybridization (2x-4x crosses). All self-pollinations were made in the greenhouse by tripping 50 flowers per head by hand using a separate toothpick on each head to trip the flowers. Flowers on three heads were selfed on each plant, with each head being manipulated on separate days. Self-fertility was determined as the number of seeds per self-pollinated flower. The number of plants representing a population ranged from 16 to 40 (average of 31).

Results

The bilaterally-derived populations produced significantly less seeds per self (0.02) than the unilaterally- (0.12), colchicine- (0.14) and nitrous oxide- (0.08) derived populations (Table 1). No differences between populations within a group were detected except for the nitrous oxide-derived group where the cultivar Vanessa produced only 0.04 seeds per self in contrast to 0.11 for Kenstar 2x4. Significant variation occurred with each population for self seed set, with the bilaterally-derived population exhibiting the lowest range. In all others the ranges within populations were similar.

The lowest percent of self-incompatible plants was in the colchicine-derived population, C21, (24.3%) and the highest in the bilaterally-derived Bi4x F_4 population (81.6%) (data not presented). When tested with the Chi-square goodness-of-fit test, the distributions of plants for percent self seed for the colchicine-, nitrous oxide- and unilaterally-derived groups were similar. However, the distribution of the bilaterally-derived material was significantly different

from the other methods with a higher frequency of plants in the self-incompatible class.

Discussion and Conclusions

When producing tetraploid red clover using chemical agents such as colchicine or nitrous oxide, the nuclear material is doubled to give twice the number of chromosomes as in the diploid. For a specific gene, say incompatibility, the diploid state would be S_1S_2, but the tetraploid state would be $S_1S_1S_2S_2$, or two doses of the two diploid alleles. There is substantial self fertility in tetraploid plants derived from chemical agents, and apparently this duplication of alleles disrupts the incompatibility system of the chemically-derived tetraploids, thus allowing self fertility to occur. In the diploid only one dose of each allele is present and self-incompatibility prevails. In addition, inbreeding occurs since the tetraploid has two copies of each diploid allele. The results of inbreeding are reduced vigor and less productive plants.

Sexually induced tetraploids are produced by the union of gametes in which one gamete (unilateral) or both gametes (bilateral) contribute the sporophytic chromosome number (2n). For example, in the bilateral (2x-2x) the cross of a S_1S_2 plant with a S_3S_4 plant would produce a tetraploid, $S_1S_2S_3S_4$, which does not contain any duplicate alleles and would be self-incompatible similar to the diploid parents. This hypothesis is supported by the high degree of self-incompatibility observed in the bilateral populations in this study. Also, sexually derived tetraploids are not inbred and should exhibit greater vigor and production than chemically-derived tetraploids. This is yet to be determined.

Table 1. Self seed set in sexually- and chemically-derived tetraploid red clover.

Doubl. meth./ Population	Seeds per self Mean	Range
	-#-	
Chemical-colchicine		
C21	0.14	0.00-0.76*
Temara	0.12	0.00-0.53*
Sally	0.17	0.00-0.66*
MEAN	0.14A+	- -
Chemical-nitrous oxide		
Kenstar 4x	0.14a++	0.00-0.45*
Vanessa	0.04b	0.00-0.35*
MEAN	0.08AB	- -
Sexual-bilateral (2x-2x)		
Bi4x F4	0.01	0.00-0.12*
Bi4x F2	0.05	0.00-0.21*
MEAN	0.02B	- -
Sexual-unilateral (2x-4x)		
TW2x4	0.11	0.00-0.65*
Ken 2x4	0.12	0.00-0.52*
MEAN	0.12A	- -

+Means between methods followed by different letters are significantly different at 5% level.
++Means between populations within a method followed by different letters are significantly different at 5% level.
* Significant variation among genotypes with a population.

SELECTION IN RED CLOVER FOR RESISTANCE TO NORTHERN ANTHRACNOSE

N.L. TAYLOR, R.R. SMITH and J.A. ANDERSON

Introduction

Northern anthracnose (NA) is a destructive disease of red clover in the temperate regions of the world. Losses exceeding 50% of the crop have been reported. Vertical resistance to NA has been reported to be dominant and controlled by two or more genes. Recurrent selection, coupled with progeny testing, has been suggested as a desirable method of breeding for resistance. The objectives of our study were to test the feasibility of developing NA resistance in Kenstar-related populations by phenotypic recurrent selection, and to determine the effectiveness of this selection procedure for populations low in variation for NA resistance.

Materials and Methods

Screening and selection procedures: Seeds of 10 modified populations derived from the clones which constitute the cultivar Kenstar and one entry of each Kenstar and NA-resistant population WI-2 were sown in greenhouse soil benches in Madison, WI. In each cycle, the plants were inoculated with a mixture of *Kabatiella caulivora* (causal agent of NA) isolates collected from naturally infected plants in Wisconsin and Kentucky and maintained on culture media in Wisconsin. Plants were evaluated using a Disease Severity Index (DSI) of 1 = no visible symptoms (resistant) to 5 = necrosis of the leaflets and lesions on the petiole (susceptible). After each of the six cycles of selection, resistant plants (usually DSI 1 or 2) were transplanted to isolation cages in Kentucky and each population intercrossed to produce seed for the next cycle of selection.

Evaluation of progress from selection: For evaluation of the phenotypic recurrent selection program, remnant seed from each of six cycles of selection for each population and the original populations were sown into greenhouse soil benches in split plot design with four replications. Plants were inoculated with *K. caulivora* and evaluated for resistance using the DSI as described above. Mean DSI/population/cycle and percent plants in the DSI 1 and 2 class were calculated.

Results and Discussion

Populations differed significantly in DSI after six cycles of selection, and the decrease in DSI over cycles was linear ($P<0.01$) (Table 1). The average DSI was reduced from 4.14 in the base material (Cycle 0) to 2.67 in Cycle 6. Individual populations were quite variable for their initial DSI (2.87 to 4.72) and for their response to the selection program. For example, Population 4 was intermediately susceptible in Cycle 0 (DSI = 2.87) and moderately resistant (DSI = 2.30) in Cycle 6. Population 5 began as susceptible (DSI = 4.25) and was improved to moderately resistant (DSI = 2.34). Eight of the 10 populations had at least 29% more resistant plants (DSI 1 and 2) in Cycle 6 than in Cycle 0.

Conclusions

Mean DSI was reduced by 36% through the six cycles of selection and was linear across cycles. Selection improved DSI by less than 24% through six cycles in four of the populations, while average improvement of the other six was 46%. Realized heritabilities averaged 20% per cycle and were greatest among the six populations with greatest improvement in DSI. The resistance obtained from these low-variation susceptible populations indicates that phenotypic recurrent selection is an effective means of uncovering latent variation for resistance to NA.

Table 1. Mean DSI and percent resistant plants (DSI of 1 and 2) for Cycle 0 and 6 in the 10 Kenstar red clover populations.

Population	DSI score		Percent resistant	
	Cycle 0	Cycle 6	Cycle 0	Cycle 6
1	4.68	2.77**	0	45
2	4.67	2.48**	0	56
3	4.11	2.38**	6	62
4	2.87	2.30	36	51
5	4.25	2.34**	8	58
6	4.72	3.68**	0	17
7	3.54	2.77**	25	54
8	4.71	3.77**	0	12
9	4.09	2.28**	6	66
10	3.82	1.94**	11	86
Mean	4.14	2.67**	9	52

** Significant linear increase in resistance over the six cycles at the 1% level.

RECURRENT SELECTION FOR PLANT REGENERATION FROM RED CLOVER TISSUE CULTURE

K.H. QUESENBERRY and R.R. SMITH

Introduction

Most current methods for genetic transformation of plants require that the individual cells which are transformed have the capacity of being regenerated into whole plants. It has been demonstrated that species of various genera are readily regenerated into whole plants from callus culture. However, the major crop species such as maize, wheat, and many forage legumes are more difficult to regenerate from callus tissue. Whole plant regeneration from tissue culture has been demonstrated for red clover; however, the frequency of regeneration is extremely low and appears to be genotypic specific. The objectives of this research were to estimate the variability for regeneration from tissue culture in adapted red clover germplasm and to select for increased plant regeneration.

Materials and Methods

The tissue culture protocols used for this research were described by Beach and Smith (Plant Sci. Lett. 15:231-237. 1979). The initial plant material that was cultured was from the red clover cultivar, Arlington. Five cycles of phenotypic recurrent selection, or a slight modification, were applied. Hypocotyl tissue was used for explants in Cycle 1 and 2 and petiole tissue in Cycles 3, 4, and 5. The number of plants evaluated, percent plants rated 3 or greater for regeneration, and the number of plants intercrossed each cycle are presented in Table 1. Cultures were rated on the following scale: 1 = little or no callus; 2 = limited callus growth; 3 = moderate callus growth with occasional green shoots; 4 = good callus growth with frequent occurrence of

developing plants; and 5 = excellent callus growth and numerous developing plants.

To evaluate the progress from selection, remnant seed of Cycles 0, 2, 3, and 4 were tested for regeneration at the same time and under the same conditions. Thirty plants of each cycle were evaluated. Progeny from cycles 2 and 3 were evaluated on a half-sib basis to estimate heritability of regeneration.

Results and Discussion

Eight plants out of 200 original Arlington plants (4%) regenerated and were selected to produce cycle 1 progeny (Table 1). Percentage regenerating plants in the subsequent cycles 1, 2, 3, 4, and 5 were 9, 16, 20, 39, and 72, respectively. The results of evaluating remnant seed of Arlington and cycles 2, 3, and 4 are given in Table 2. The percentage of plants rating 3 or higher in this experiment was similar to the original percentages as determined during each year of selection. These results show that the random sample from each cycle to initiate each selection cycle represented the overall genetic diversity in each population and that the response of these genotypes to tissue culture was similar over environments.

Narrow sense heritability estimates of the plant regeneration response ranged from 50% for the Cycle 2 population to 40% for the Cycle 3. Although the number of half sib families evaluated in each generation was relatively small, the progress made through selection is indicative of high heritability of this trait. The results would also suggest that a relatively small number of genes likely control this response.

Conclusions

The results of this study demonstrate that the traditional phenotypic recurrent selection breeding methodology can be applied to select for increased frequency of plant regeneration from callus culture in red clover. The Cycle 5 population is currently being increased for further evaluation and a germplasm release is planned.

Table 1. Number of plants evaluated, selected and intercrossed for regeneration for each of the selection cycles.

Cycle	Plants evaluated	% of plants rated > 3	Plants intercrossed
0	200	4	8
1	100	9	8
2	80	16	13
3	140	20	26
4	170	39	26
5	50	72	—

Table 2. Evaluation of remnant seed of Arlington and Cycle 2, 3, and 4 for regeneration from tissue culture.

Population	Number plants+ > 3	Percent > 3	Percent > 3 in original selection
Arlington (C0)	1	3	4
Cycle 2	4	13	16
Cycle 3	5	17	20
Cycle 4	14	47	39

+ 30 plants of each population were evaluated.

COMPOSITION OF CELL WALLS ISOLATED FROM INDIVIDUAL CELL TYPES OF GRAIN SORGHUM STEMS

R.D. HATFIELD, J.R. WILSON and D.R. MERTENS

Introduction

The utilization of structural polysaccharides in forage cell walls by rumen microbes is incomplete and dependent upon the type of forage and maturity. Although it is generally believed that lignification of the cell wall during maturation is the major structural change leading to decreased digestibility, the responsible mechanism has not been identified. Characterization of specific tissues at defined stages of maturity has provided important insights into matrix changes and their relationships to digestibility. However, detailed structural analysis is compromised by the presence of several different cell types each with different structural/functional roles and most likely different cell walls in terms of composition and structural organization. This initial study was undertaken to investigate the digestibility differences between individual cell types and to determine the composition of the different cell walls. This is a report of the cell wall composition of five different cell types isolated from the fifth internode of grain sorghum at anthesis and grain maturity.

Methods

Grain sorghum plants were grown in Brisbane, Australia and harvested at anthesis and grain maturity stages. The fifth internode from the top was separated from individual plants and quick frozen in liquid nitrogen and stored at -20°C. While still frozen, internodes were separated by hand into different cell types and individual isolates freeze dried. Cell types isolated included epidermis, sclerenchyma, vascular bundle zone, pith, and inner vascular cells.

Cell walls were isolated from individual cell types using a modified Uppsala method. Briefly cells were suspended in 80% ethanol and sonicated for 10 min., centrifuged, and the supernatant removed. The ethanol extraction was repeated three times followed by a single wash of chloroform:methanol (2:1) and a final rinse of acetone. Air dried walls were suspended in phosphate buffer (pH 6.9) and heated to 80-90°C for 2 h. Samples were cooled to 55°C, α-amylase added, and incubated for 1h. The pH of each sample was adjusted to 5.5 and amyloglucosidase added and incubated for 1.5h. Cell wall residues were collected on glass fiber filters, washed with cold water followed by acetone, and allowed to air dry.

Cell wall residues were analyzed for total carbohydrate, total uronic acids, neutral sugar composition, and Klason lignin content. Original cell isolates were subjected to pyrolysis-GC-MS analysis to compare the lignin and phenolic acid components of the different cell walls.

Results and Discussion

Although the two harvest dates were selected to potentially provide a reasonable difference in cell wall development, the two stages were similar in gross composition (Table I). For grain sorghum, cell wall development appears to stop at anthesis. Cell wall recovery (% of DM) from the different cell types was somewhat lower at grain maturity most likely due to starch accumulation after anthesis. Gross wall composition (Table I) was similar between cells at anthesis and those at grain maturity. The major differences in the cell types within the internode would appear to be in

the total uronics and the Klason lignin values for the walls (Table I).

The neutral sugar composition of cell walls was not appreciably different between the two plant maturities. Individual cell types did show some differences in composition although the range was small (Fig.1). Pith cells which have little or no secondary wall contained higher proportions of arabinose, rhamnose, galactose, and xylose. Sclerenchyma, inner vascular bundles (IVB), and vascular bundle zone (VBZ) cell walls have high amounts of secondary thickening and were enriched in glucose with low amounts of uronics. The epidermal cells were the most distinct with neutral sugar composition similar to pith cells; however, they were low in total uronics and high in lignin. The high lignin values are due to cuticular material which is a composite of waxes and some phenylpropanoid material.

Pyrolysis-GC-MS analysis of the different cell types indicated similar lignin compositions. For pith, sclerenchyma, IVB, and VBZ cell types, the guaiacyl to syringyl ratio ranged from 1.46 to 1.53 indicating a close similarity in lignin. Epidermal cells were much different with a ratio of 2.41.

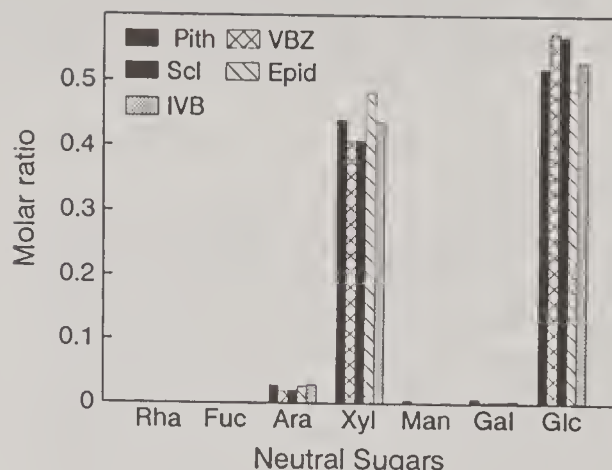


Figure 1. Neutral sugar composition of cell walls isolated from pith (Pith), sclerenchyma (Scl), vascular bundle zone (VBZ), inner vascular bundles (IVB), and epidermal (Epid) cells of grain sorghum stems harvested at anthesis.

Larger differences were seen in the ratio of *p*-coumaric acid (*p*-CA) to ferulic acid (FA) (Table I). The pith cells contained the lowest amounts of FA with *p*CA levels similar to other cell walls. Epidermal and pith cell walls appear to have the most unique compositions with the other cell types being more or less grouped together.

Table 1. Recovery and composition of cell walls isolated from the 5th internode of grain sorghum.

		g/ g Cell wall				
	Cell Wall % of DM	Total Uronics	Total NS[c]	Klason Lignin	Total Recovery	*p*-CA/FA Ratio
Ant.[a] Pith Cells	25.44	0.026	0.720	0.170	0.916	5.23
GM.[b] Pith Cells	17.51	0.027	0.767	0.168	0.962	5.11
Ant. VBZ	75.97	0.013	0.754	0.179	0.945	1.70
GM. VBZ	69.97	0.014	0.822	0.202	1.038	2.23
Ant. Scl.	70.48	0.010	0.764	0.202	0.977	1.23
GM. Scl.	70.32	0.012	0.730	0.213	0.955	1.17
Ant. Epidermis	75.43	0.007	0.764	0.218	0.988	0.58
GM. Epidermis	86.18	0.007	0.742	0.218	0.967	0.48
Ant. IVB	58.88	0.018	0.750	0.188	0.955	2.40
GM. IVB	46.31	0.018	0.743	0.197	0.958	2.12

[a]Cells at anthesis stage [b]Cells at grain maturity [c]NS=neutral sugars

DIFFERENCES IN DIGESTION KINETICS AND INDIGESTIBLE RESIDUE OF ISOLATED CELL TYPES FROM SORGHUM STEMS

D.R. MERTENS, J.R. WILSON (CSIRO, Australia) and R.D. HATFIELD

Introduction

Interpretation of digestion kinetics of plant cell walls (CW) is difficult in whole forage samples because the process represents the average digestion of numerous cell types. Visual evidence suggests that parenchyma and mesophyll CW digest more rapidly than those in vascular bundles or sclerenchyma. Therefore, the relationships between digestion kinetics and chemical characteristics that limit digestion will be more easily elucidated by investigating individual cell types. The objective of this study was to test this hypothesis by identifying differences in chemical composition and digestion kinetics of cell types isolated from sorghum stems.

Materials and Methods

Sorghum was selected as a model plant for isolating cell types because the size of morphological components allows separation of tissues more easily. The fifth internode of sorghum stems was harvested at anthesis and grain maturity stages of development and tissues were separated by a manual technique developed in Dr. Wilson's laboratory. Cell types were classified into epidermis, sclerenchyma (SCL), vascular bundle zone (VBZ), inner vascular bundles (IVB) and pith. Due to limited quantities of epidermis material, digestion kinetics and detergent analysis were determined only on the latter four cell types. Cell wall preparations and Klason lignin analysis were determined by methods of Hatfield (described in another report). Neutral detergent fiber (NDF) was determined using sodium sulfite and amylase, and results are reported on an ash-free basis. Acid detergent fiber (ADF) and sulfuric acid lignin were determined by standard procedures.

Digestion kinetics of NDF were determined in two runs using the in vitro method of Goering and Van Soest. Due to the small amount of isolates available, samples were fermented for 0, 6, 12, 24, 48 and 96 h in the first trial and 0, 3, 6, 9, 12, 24, 30, 48 and 96 h in the second in vitro trial. Nonlinear regression was used to fit the data to the model:

$$R(t) = Do^{*}e^{(-kd^{*}[t-L])} + I;$$

where R(t) is the NDF residue remaining at each time, Do is the potentially digestible fraction, kd is the first-order fractional rate constant of digestion, t is time after inoculation, L is discrete lag time and I is the indigestible residue.

Results and Discussion

There was little change in digestion kinetics between anthesis and grain maturity in cells obtained from the fifth internode of sorghum stems (Table 1), nor were there differences in chemical composition between maturities (Table 2). This indicates that changes in CW characteristics occur before anthesis, with little change thereafter. In addition, it suggests that changes in whole plant composition and digestion kinetics may be due to changes in proportions of cell types rather than gradual changes in the CW of all tissues in the plant. The only characteristic that varied with maturity was NDF or CW concentration in pith, IVB and VBZ which decreased with maturity as storage carbohydrates accumulated in the stems.

The only differences in digestion kinetics between cell types were longer lag times for VBZ, faster fractional rates for SCL and greater indigestible residues as a percentage of NDF for pith. Longer lag for VBZ indicates that bacterial attachment may limit digestion in cell types which have long cylindrical cell structures. The faster rate of digestion of SCL in this study does not agree with visual observations that suggest these cells are digested slowly. It was

unexpected that the pith cells, with the least amount of lignin in DM or CW, would have the largest fraction of NDF be indigestible. This indicates that factors other than lignin may play a role in protecting CW's from digestion. Although there are large differences in lignin concentration between Klason and AD lignin, values were highly correlated with each other and with indigestibility as a fraction of DM. Agreement between concentrations of CW and NDF was excellent.

Conclusions

Isolated cell types differ in digestion kinetics and relationships between digestion and chemical composition. They provide a useful tool for basic investigations into chemical and physical factors that limit digestion, because relationships are not confounded by differing proportions of cell types as occurs in whole plant tissue.

Table 1. Parameters of digestion kinetics for isolated cell fractions from sorghum stems. Digestible and indigestible fractions are expressed as percentages of NDF and g/kg dry matter.

Cell Isolate Maturity	Discrete lag	Fractional rate	Potentially digestible		Indigestible residue	
	(h)	(h^{-1})	(g/kg)	(%)	(g/kg)	(%)
Pith cells						
Anthesis	4.90	.040	119	(48)	127	(52)
Grain maturity	4.96	.033	95	(55)	77	(45)
Inner vascular bundle cells						
Anthesis	3.80	.034	424	(75)	138	(25)
Grain maturity	3.99	.032	367	(75)	120	(25)
Sclerenchyma cells						
Anthesis	4.70	.064	423	(61)	276	(39)
Grain maturity	3.67	.061	427	(61)	271	(39)
Vascular bundle zone cells						
Anthesis	7.89	.040	531	(70)	223	(30)
Grain maturity	7.25	.030	476	(67)	233	(33)

Table 2. Chemical composition of isolated cell types from sorghum stems. Klason lignin is the cell wall (CW) residue remaining after solubilization with 72% sulfuric acid followed by hydrolysis in 1.6M sulfuric acid for 3h. Acid detergent (AD) lignin is the residue remaining after extracting with AD (1M sulfuric acid) then solubilizing with 72% sulfuric acid.

Cell type Maturity	Cell walls in DM	Klason lignin in DM	Klason lignin in CW	NDF in DM	AD lignin in DM	AD lignin in NDF
	(g/kg)					
Pith cells						
Anthesis	254	43	170	249	4	15
Grain maturity	175	29	168	173	1	8
Inner vascular bundle cells						
Anthesis	588	111	188	573	14	25
Grain maturity	463	91	197	497	12	24
Sclerenchyma cells						
Anthesis	705	143	203	707	34	48
Grain maturity	703	151	214	708	31	44
Vascular bundle zone cells						
Anthesis	760	136	179	764	35	45
Grain maturity	700	142	203	718	32	44

A COMPARISON OF THE INSOLUBLE RESIDUES PRODUCED BY THE KLASON LIGNIN AND ACID DETERGENT LIGNIN PROCEDURES

R.D. HATFIELD, H.J. JUNG, J. RALPH, D.R. BUXTON and P.J. WEIMER

Introduction

Lignin determination in forage samples has predominately been the acid detergent lignin method developed by Van Soest. The Klason lignin procedure was developed for use with woody species and is still used for these samples today. It is generally thought that the Klason procedure is not appropriate for forages, especially legumes, due to the higher levels of protein found in these plants. The advantage of the Klason procedure is that it can be easily adapted to a cell wall hydrolysis scheme in order to obtain values for total carbohydrates, total uronic acids, and neutral sugar composition of forage samples. This work was undertaken to investigate the chemical differences in the insoluble residues (lignin) created by the two methods.

Methods

Alfalfa, orchardgrass, and switchgrass were harvested at three maturity stages, dried, and separated into stems and leaves. Only stem material was used in this investigation. All samples were ground through a 0.5 mm Udy

mill. Lignin assays were based on the method of Van Soest (Goering and VanSoest, 1975) for ADL determinations and the Uppsala method (Theander and Westerlund, 1986) for Klason lignin determinations. Additional treatments included varying the secondary hydrolysis time for Klason lignins, addition of protein, lysine, or ammonium sulfate to samples, and pretreatment of samples with neutral detergent as compared to alcohol extraction. Analysis of the insoluble residues generated by each procedure included dry matter recovery, total nitrogen, total ash, and pyrolysis-GC-MS.

Results and Discussion

There was a significant range in NDF recovery from the different plant stems as expected. Maturity resulted in increased amounts of NDF for alfalfa and orchardgrass stems but appeared to have little effect on switchgrass stems (Table 1). Lignin concentrations increased with maturity with either lignin assay procedure (Table 1). Values obtained by the Klason procedure were consistently higher than those obtained by the ADL procedure. Although the total nitrogen concentration was higher in the Klason lignin samples (Table 2), the difference was not sufficient to account for the large differences seen in the grass samples (Table 1). One would have expected the alfalfa samples to have even greater differences due to higher crude protein contents. Addition of nitrogen containing compounds (bovine serum albumin, lysine, and ammonium sulfate) to cellulose and arabinoxylans that were subjected to the Klason lignin procedure did not increase the insoluble residue over the control. This would indicate that increasing amounts of protein do not result in an automatic increase in the Klason residue. It is interesting to note that the ADL residues from alfalfa also contained higher levels of residual nitrogen. Perhaps this represents wall bound protein that cannot be extracted. Pretreatment of samples with ND solution resulted in a slightly lower Klason residue as compared to the normal procedure. ADL residues were not affected by the ND pretreatment.

A possible point in the Klason procedure that could result in an increase in lignin residue would be the secondary hydrolysis step. Varying the hydrolysis time from 0.5 to 3 h changed the amount of lignin residue recovered. Hydrolysis times of 0.5 h increased the residues indicative of incomplete hydrolysis of complex carbohydrates. Longer times also increased the residue which was most likely due to degradation of sugar monomers to furfural derivatives with subsequent polymerization into acid insoluble material. For all forage samples the optimum hydrolysis time was 1h as developed in the original procedure.

Pyrolysis-GC-MS analysis of the original plant materials, ADL and Klason lignin residues revealed a large amount of similarity among the samples. In terms of major peaks derived from lignin, their abundance relative to guaiacol did not change appreciably. ADL and Klason lignin residues differed only in 4-vinylphenol (derived from *p*-coumaric acid) and 4-vinylguaiacol (derived from ferulic acid) compounds. The ADL residues were consistently lower than the Klason lignin residues for these two compounds. It is not possible to explain the differences as loss of phenolic acids during the ADL procedure since the total amounts in the original samples are not sufficient to account for the mass difference.

Conclusions

The differences in ADL and Klason lignin residues could not be explained totally by increased precipitation of protein. If this were the case, one would have expected the alfalfa samples to have greatest differences. The lignin residues appear to be chemically similar although for grasses the amount of ADL is lower by a factor of 3 to 4. The lower values for grass ADL residues would suggest that a portion of the lignin may be acid soluble and is efficiently removed by the AD treatment.

Table 1. Concentration of neutral detergent fiber (NDF), crude protein (CP), acid detergent lignin (ADL), and Klason lignin (KL) in forage stem samples.

Species	Maturity	NDF	CP	ADL	KL
		% Dry Matter			
Alfalfa	Early	52.5	10.7	8.2	11.0
	Mid	63.5	8.6	10.7	14.8
	Late	66.0	7.5	11.7	15.2
Orchardgrass	Early	54.1	5.8	1.5	5.3
	Mid	65.7	3.3	4.1	11.1
	Late	61.0	2.3	4.5	13.2
Switchgrass	Early	75.1	2.7	5.4	12.7
	Mid	74.3	1.8	7.0	14.4
	Late	76.0	1.4	7.5	14.5

Table 2. Nitrogen concentration of Klason lignin (KL) and acid detergent lignin (ADL) preparations from forage stem samples.

Species	Lignin	Maturity		
	Method	Early	Mid	Late
Alfalfa	KL	1.84	1.72	1.74
	ADL	1.18	1.31	1.27
Orchardgrass	KL	1.83	1.40	1.42
	ADL	0.93	0.67	0.66
Switchgrass	KL	1.18	1.04	0.92
	ADL	0.53	0.49	0.50

IMPACT OF A PHENOLIC ACID-SUGAR COMPLEX ON POLYSACCHARIDE DEGRADATION

D.A. DEETZ, H.G. JUNG, R.F. HELM, R.D. HATFIELD and J. RALPH

Introduction

Phenolic acids esterified to forage cell wall polysaccharides are solubilized by enzymatic hydrolysis of the polysaccharides. The resultant soluble complexes have been shown to consist of ferulic and *p*-coumaric acids esterified to sugar dimers and trimers (arabinose-xylose and arabinose-xylose-xylose, respectively). Numerous reports of the potential toxicity of free phenolic acids to polysaccharide degradation by rumen microorganisms have been published. Synthetic methyl- and butyl-esters of *p*-coumaric acid have also been shown to inhibit forage fiber digestion. However, these toxic effects are only seen at very high concentrations. In contrast, esterification of phenolic acids to arabinoxylan polymers inhibited degradation at much lower concentrations. The objective of this study was to examine the potential toxicity of a more realistic model of cell wall phenolic acid-sugar complexes.

Materials and Methods

The synthetic model compound methyl 5-*0*-*trans*-feruloyl-α-L-arabinofuranoside (FA-Ara) was included in *in vitro* ruminal fermentations of microcrystalline cellulose and oat spelts xylan. Treatments included 0 (Control), 15, 150, 1500 and 4500 μ*M* concentrations of methyl α-L-arabinofuranoside (Me-Ara), ferulic acid (FA), and FA-Ara in the fermentation media. Degradabilities of glucose from cellulose, and xylose and arabinose from oat spelts xylan were determined after 24 and 72 h *in vitro* fermentations of the individual polysaccharides. Survival of FA-Ara following the incubation was estimated by absorbance at 375 nm.

Results and Discussion

Table 1 summarizes the observed *in vitro* degradabilities of the polysaccharides. Addition of the FA-Ara significantly depressed glucose and arabinose degradabilities at 24 h of fermentation, but the concentrations at which these effects were observed were not consistent. Degradability of all polysaccharide components after 72 h of incubation was not affected by FA-Ara addition to the media. The negative effect of FA-Ara appears to be a result of the Me-Ara component of the complex as Me-Ara alone also depressed 24 h glucose and arabinose degradabilities. Again, this effect was not seen after 72 h or for xylose degradation. The transient negative effect of Me-Ara was probably the result of reduced polysaccharidase synthesis by the microorganisms while the more readily available sugar substrate was present. That this effect was greater for arabinose degradation from the oat spelts xylan might be expected because Me-Ara is an analog of arabinose and is probably metabolized similarly by rumen microorganisms.

Free FA only inhibited 24 h glucose degradation from cellulose at the highest concentration tested. This is similar to results reported previously for the negative effects of FA. Ferulic acid has also been reported to stimulate polysaccharide degradation in some cases. This was seen in our study for 24 h arabinose degradation from oat spelts xylan. This result may be a reflection of the requirement of *Ruminococcus albus* for 3-phenylpropionic acid which is a ruminal degradation product of phenolic acids.

Very little FA-Ara was found in fermentations after 24 h and this complex was completely absent after 72 h. These results were seen at all levels of FA-Ara addition. These data support previous reports that FA is rapidly degraded by many rumen microorganisms and that feruloyl-esterase activity is high in the rumen. We conclude that FA-Ara is not toxic to rumen microorganisms and that the inhibitory effects of phenolic acids esterified to arabinoxylans in grasses are limited to steric hindrance of polysaccharidases. This inhibition is expected to affect rate of cell wall degradation, but not ultimate extent.

Table 1. Degradation of neutral sugars from isolated polysaccharides in the presence of FA-Ara.

		Cellulose		Oat Spelts Xylan			
		Glucose		Xylose		Arabinose	
Treatment	Concentration	24h	72h	24h	72h	24h	72h
	µM	g kg^{-1}					
Control	0	376	852	296	717	513	846
FA-Ara	15	318	823	330	679	546	841
	150	307[a]	871	297	778	493	871
	1500	389	827	306	740	374[a]	854
	4500	380	833	415	661	182[a]	811
Me-Ara	15	361	919	285	724	505	854
	150	332	847	229	712	469	867
	1500	310[a]	832	273	716	309[a]	850
	4500	320[a]	836	341	672	160[a]	792
FA	15	381	870	377	745	604[b]	854
	150	350	930	365	775	601[b]	871
	1500	345	912	362	823	547	888
	4500	326[a]	845	527	795	653[b]	855

[a]Significantly less than control (*P*< 0.05).
[b]Significantly greater than control (*P*< 0.05).

DEGRADATION OF CELL WALL POLYSACCHARIDES FROM DIVERGENTLY SELECTED ALFALFA

H.G. JUNG and R.R. SMITH

Introduction

Production of perennial forage crops is generally regarded as being more environmentally benign than annual row crops because of the lower inputs of fertilizer and pesticides and reduced soil erosion with forages. Incorporation of more forage in dairy production rations, however, is limited by the reduced available energy content of forages compared to grains. The cause of this poor energy value of forages is related to their high content of cell wall material and its poor degradability in the rumen. Increased forage utilization in dairy production will require genetic improvement of forages by reducing their cell wall content and/or increasing cell wall degradability. The objective of this study was to evaluate the response of cell wall polysaccharide degradation in alfalfa following selection for two different criteria.

Materials and Methods

Alfalfa had been divergently selected for high and low 72% acid detergent lignin (ADL) content in whole herbage by R. R. Hill at the U. S. Pasture Laboratory. These high and low ADL populations were then subsequently selected for high and low 48 h *in vitro* dry matter disappearance (IVDMD) of stems. Selection for IVDMD changed stem degradability of both ADL popula-

tions. Basal stem samples were collected from polycross progeny of the four populations (high ADL:low IVDMD, high ADL:high IVDMD, low ADL:high IVDMD, and low ADL:low IVDMD) at bud stage from two cuttings. Degradability of cell wall neutral sugars and uronic acids after 48 h *in vitro* ruminal fermentations was determined.

Results and Discussion

Table 1 summarizes the degradability of polysaccharides from divergently selected alfalfa. Cellulose degradation, estimated as glucose degradation from the cell wall, was not affected by selection for either ADL or IVDMD. In contrast, degradation of non-cellulosic polysaccharides was greater for low ADL populations than for high ADL populations at both cuttings. Selection for IVDMD, subsequent to the ADL selection, did not affect degradation of the non-cellulosic polysaccharides. All of the sugar components of the non-cellulosic polysaccharides (fucose, rhamnose, arabinose, galactose, xylose and uronic acids) exhibited this response to ADL selection, but not IVDMD, except for mannose. Mannose degradation was unaffected by either selection regime.

Interestingly, selection for ADL resulted in reduced neutral detergent fiber (NDF) content of the low ADL populations, but total cell wall content was not altered. The IVDMD selected populations did not differ in NDF or cell wall content. These results suggest that selection for low ADL resulted in a less lignified cell wall and that a larger proportion of the cell wall was soluble in neutral detergent. The study indicates that selection for lignin may be a more powerful tool for improving the genetic potential of forages for cell wall polysaccharide digestion than is selection for IVDMD.

Table 1. Cell wall polysaccharide degradability in divergently selected alfalfa.

	Cellulose		Non-Cellulosic Polysaccharides	
Populations	Cut 1	Cut 2	Cut 1	Cut 2
	$g\ kg^{-1}$			
High Lignin	510	481	467*	497*
Low Lignin	516	517	498	546
High IVDMD	508	481	481	505
Low IVDMD	518	522	484	538

*High and low populations, within a selection criteria, differ ($P < 0.05$).

CHEMICAL COMPOSITION OF INDIGESTIBLE RESIDUES FROM FORAGES

D.R. MERTENS

Introduction

A portion of the cell wall of plants is indigestible by animals. Computer models of digestion and passage in ruminants suggest that this fraction is a key factor limiting the intake and utilization of forages. Although the existence of an indigestible fraction has been documented in in vitro and in situ fermentations, little research has been done to characterize this fraction as a means of

identifying factors which limit digestion and intake of forages by dairy cows. The objectives of this project were: to determine if an in vitro procedure could isolate indigestible residues with characteristics similar to those found in animal feces, to evaluate the effects of pre-extraction with neutral detergent (ND) on subsequent chemical analyses and to test the hypothesis that indigestible residues have a constant proportion of lignin regardless of the source.

Materials and Methods

Six forages (alfalfa and red clover to represent legumes, orchardgrass and brome to represent C3 or cool season grasses and corn stalks and big bluestem to represent C4 or warm season grasses) were selected which had been fed to sheep during a digestion trial. A composite sample of feces from four sheep fed at 1X maintenance level of intake was used to obtain indigestible residues after animal digestion. The in vitro procedure of Goering and Van Soest was used to isolate indigestible residues from forages and respective feces. Feeds were replicated 18 times and feces were replicated 6 times within each in vitro run. In vitro runs were replicated four times. After incubation for 168 h, in vitro residues were freeze-dried, composited and reground using a UDY cyclone mill with a 1 mm screen.

Neutral detergent fiber (NDF) was determined using sodium sulfite and amylase and residues were filtered on Whatman GF/D glass microfibre mats in fritted disc crucibles to retain fine particles. Due to the high ash content of in vitro indigestible residues, results were expressed on an ash-free, organic matter basis. Routine acid detergent fiber (ADF) and acid insoluble lignin (ADL) were analyzed by the methods of Goering and Van Soest. In addition, ADF and ADL were determined on residues after sequential extraction with neutral detergent (sADF; sADL).

Results and Discussion

With the exception of the very immature orchardgrass sample, indigestible residues were similar when the starting material was the forage or was feces obtained from animals fed the forage (Table 1). This indicates that the in vitro method can be used to isolate indigestible residues from feeds in future investigations concerning the characteristics of indigestible cell walls. By starting with feces, the recovery of indigestible residues is effectively doubled.

Pre-extraction of residues with ND had a significant impact on recoveries of ADF and ADL (Table 1). Recoveries of sADF and sADL were only 87 and 76% of ADF and ADL, respectively for legumes and these recoveries were lower for grasses (79 and 63%, respectively). It is unknown what components ND could extract from indigestible residues that would not be removed by routine ADF analysis. It is speculated that these unknown compounds may be phenolics or tannins that are solubilized by ND, but precipitated by acid during ADF analysis.

The hypothesis that indigestible residues have similar lignin concentrations is refuted by the analytical results of C4 grasses. Although legumes and C3 grasses in this study had similar ADL and sADL concentrations, their concentrations were lower in C4 grasses. Assuming the difference between NDF and ADF is a measure of hemicellulose, it appears that indigestible NDF of grasses contain more hemicellulose than that of legumes.

Conclusions

In vitro procedures can be used to isolate indigestible fractions that are similar to those obtained by ruminant digestion. Sequential extraction of ADF and ADL result in lower values than those determined by routine methods; however, regardless of extraction procedure, indigestible residues do not contain a constant proportion of lignin.

Table 1. Chemical composition of in vitro indigestible residues after 168 h of fermentation. Neutral detergent fiber (NDF) is expressed as a percentage of organic matter (OM), while routine and sequential acid detergent fiber (ADF; sADF) and acid detergent lignin (ADL; sADL) are expressed as a percentage of NDF.

Type	Forage	Source	NDF	ADF	sADF	ADL	sADL
			(%OM)	----------(%NDF)---------			
Legume	Red clover	Feed	22.3	87.3	71.4	30.1	20.9
Legume	Red clover	Feces	42.6	89.3	74.2	31.5	21.8
Legume	Alfalfa	Feed	33.1	81.0	76.0	30.3	26.3
Legume	Alfalfa	Feces	50.0	84.7	76.5	31.5	25.3
C3 Grass	Orchardgrass	Feed	7.5	77.8	56.9	42.5	29.0
C3 Grass	Orchardgrass	Feces	19.3	65.0	48.8	28.6	16.8
C3 Grass	Brome	Feed	13.6	69.9	52.5	25.1	15.0
C3 Grass	Brome	Feces	35.2	60.5	50.8	19.2	12.4
C4 Grass	Corn stalks	Feed	25.8	66.5	55.3	15.9	9.1
C4 Grass	Corn stalks	Feces	38.0	63.4	55.0	13.2	8.4
C4 Grass	Big bluestem	Feed	19.2	61.5	47.5	15.6	9.4
C4 Grass	Big bluestem	Feces	36.5	62.5	49.1	14.3	9.9
Legume	Average		37.0	85.6	74.5	30.9	23.6
C3 Grass	Average		18.9	68.3	52.3	28.9	18.3
C4 Grass	Average		29.9	63.5	51.7	14.8	9.2

SYNTHESIS OF HYDROXYCINNAMOYLATED ARABINOXYLAN DISACCHARIDES

R.F. HELM and J. RALPH

Introduction

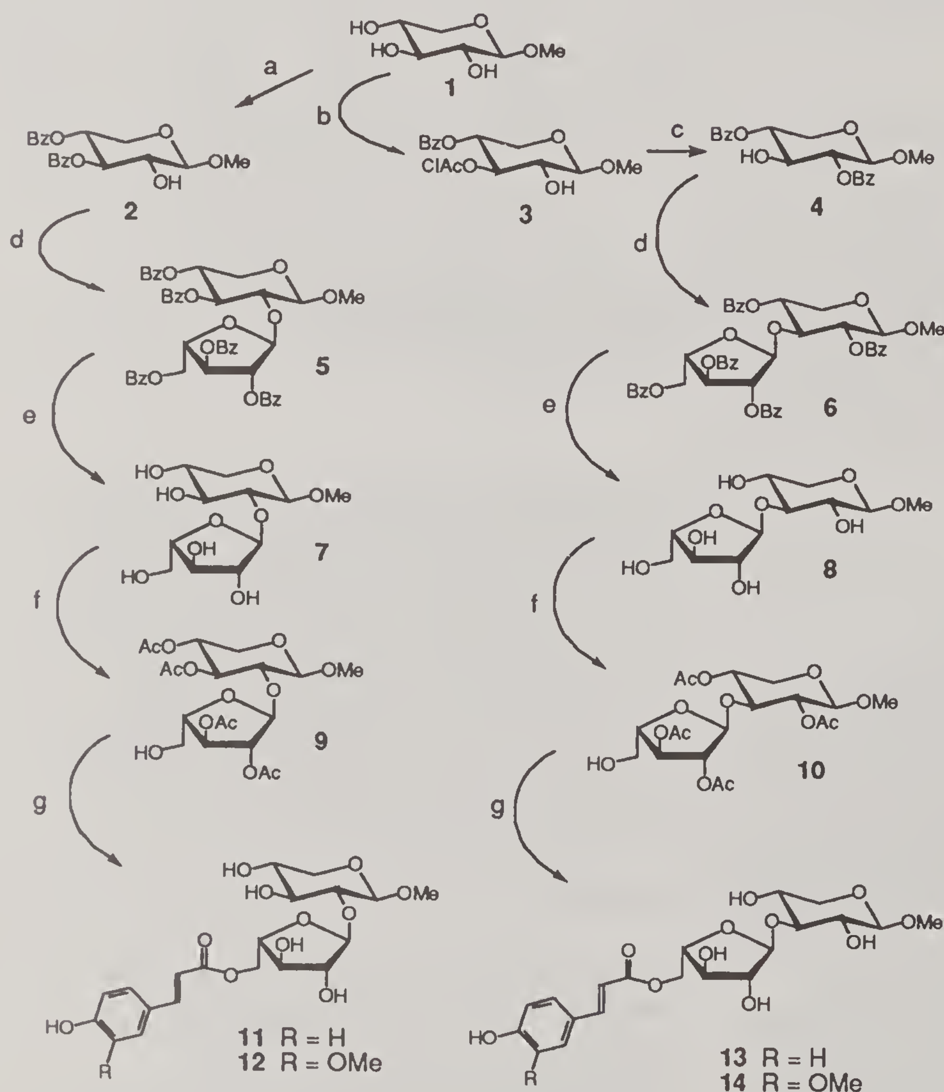
This research project is part of a larger study concerned with discerning the regiochemistry of attachment of hydroxycinnamic acids (ferulic and *p*-coumaric acids) to lignin and hemicellulose in forage plant cell-walls. This information is vital to our understanding of the biochemical aspects of cell-wall synthesis and degradation. Our use of NMR spectroscopic techniques for the elucidation of this bridging phenomenon requires suitable model compounds with which to optimize the spectroscopic conditions used for the analysis of native tissue. These compounds can also be used as substrates in enzymatic studies concerned with the degradation of cell-wall components. It is also hoped that eventually antibodies can be generated against some of these models so that the location of specific bonding patterns in whole cell tissue can be visually observed by microscopic methods. Procedures have been developed to prepare up to gram quantities of hydroxycinnamoylated arabinoxylan disaccharides in high yields and a brief description of the procedures used are summarized here.

Methods

Dibutyltin oxide-mediated acylation of methyl β-D-xylopyranoside (**1**, Scheme I) with benzoyl chloride (2 equiv) gave **2** (Scheme I, step a) which could be glycosylated directly with 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl chloride to form the perbenzoylated disaccharide **5**.

Treatment of **1** with dibutyltin oxide followed by benzoyl chloride (1 equiv) and subsequently chloroacetyl chloride (1 equiv) gave crystalline **3** in 54% yield. Benzoylation of **3** followed by selective removal of the chloroacetate group with thiourea gave **4** in 85% yield from **3**. Glycosylation of **4** gave **6** in 40% overall yield from **1**. Standard debenzoylation of protected disaccharides **5** and **6** (NaOMe/MeOH) gave the free disaccharides **7** and **8**, respectively, in over 90% yield.

Selective insertion of the hydroxycinnamoyl moiety at the 5-position of the L-arabinofuranosyl ring was accomplished by taking advantage of the fact this is the only primary hydroxyl in the molecule. Silylation with the bulky *t*-butyldimethylsilyl chloride in pyridine occurred exclusively at the desired 5-position. The addition of acetic anhydride to the mixture (once silylation was complete) gave the fully protected material which was selectively desilylated with aqueous 80% acetic acid. This three-step reaction sequence afforded compounds **9** and **10** in 75-80% overall yield based on the unprotected disaccharides **7** and **8**. Subsequent acylation with 4-acetoxyhydroxycinnamoyl chloride in pyridine gave the peracetylated hydroxycinnamoyl arabinoxylan disaccharides in over 90% yield. Removal of the acetate groups with pyrrolidine in 95% ethanol afforded the desired materials **11** - **14** in 80% yield.



Scheme I. Synthetic pathway to the hydroxycinnamoylated arabinoxylan disaccharides.

Discussion

The key transformation for the preparation of the (1→2)-linked materials is the one-step synthesis of **2**, the disaccharide precursor. As for the (1→3)-linked compounds, the use of N,N-(diisopropyl)ethylamine/4-(dimethylamino)pyridine proved indispensible for the insertion of the benzoyl group at the 2-position of **3** without modifying the chloroacetate group. Subequent controlled removal of the chloroacetate group gave the desired disaccharide precursor. The attachment of the hydroxycinnamoyl group to the 5-position of the α-L-arabinofuranosyl moiety proceeded according to our previously developed procedures which suggests that even higher arabinoxylans may be selectively hydroxycinnamoylated by this reaction sequence.

Conclusion

A reaction sequence for the preparation of methyl (5-*O*-feruloyl-α-L-arabinofuranosyl)-(1→3)-β-D-xylopyranoside, the companion 5-*O*-*p*-coumaroyl disaccharide, and their (1→2) analogs have been developed. The (1→2) and (1→3) hydroxycinnamoylated disaccharides are available in 9 and 11 steps, respectively, from L-arabinose and methyl β-D-xylopyranoside. Complete spectral characterization unambigously confirms assignments made from analogous materials isolated from plant cell-walls.

MODELS OF THE LIGNIN-FERULIC ACID-CARBOHYDRATE CROSSLINK

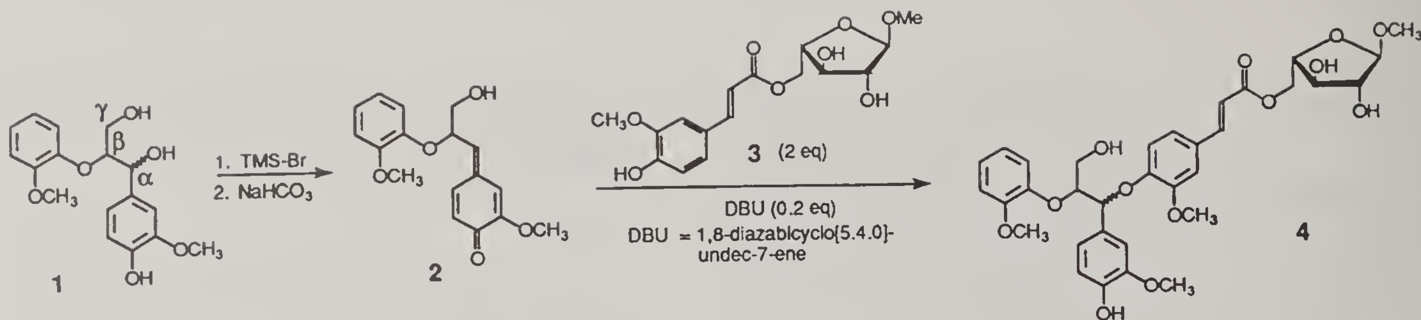
R.F. HELM and J. RALPH

Introduction

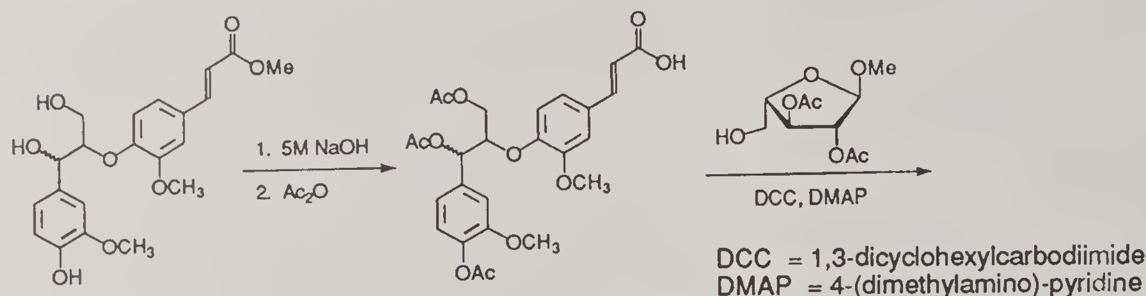
An intriguing aspect of forage cell-walls is the proposed covalent interaction among lignin, hydroxycinnamic acids (*p*-coumaric and ferulic acids), and polysaccharides. Although strong direct evidence for this crosslink is currently lacking, this bridging phenomenon has been proposed as a mechanism by which cell-wall degradation is inhibited by ruminants. The presence of relatively few crosslinks can obviously have a tremendous impact on the three-dimensional nature of the cell-wall, providing covalent bonds that cannot be enzymatically hydrolyzed, and thereby regions of restricted access where hydrolytic enzymes cannot penetrate. It is important to identify and quantitate these interactions so as to gain insight into the underlying mechanisms of cell-wall biosynthesis and degradation. Only at this point can directed studies be undertaken in efforts to improve ruminant digestion efficiency. In this context procedures have been developed to prepare two synthetic models of the lignin-hydroxycinnamic acid-polysaccharide interaction which serve as spectroscopic tools for observing these same linkages in forage tissue.

Methods

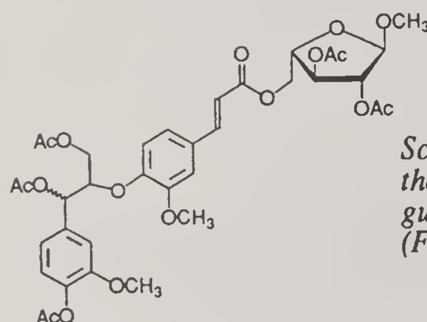
Preparation of the α-ether (**4**) was accomplished using classical lignin chemistry reactions (Scheme I). The guaiacylglycerol-β-guaiacyl ether model (**1**) was converted the the quinone methide (**2**) and subsequent exposure to **3** under basic conditions provided an equilibrium mixture of starting materials and the α-ethers (**4**), with a *threo/erythro* mixture being isolated in a 16% yield. The synthesis of the β-substituted material (**7**) required a sequence of modifications to the previously prepared β-feruoyl ether model (**5**, Scheme II). Removal of the methyl ester group was accomplished by treatment with NaOH in dioxane/water. The free acid was subsequently acetylated with acetic anhydride/pyridine to afford the protected free acid **6**. The attachment of the α-L-arabinofuranosyl moiety was achieved



Scheme I. Preparation of the FA-Ara α-ether of guaiacylglycerol-β-guaiacyl ether.



DCC = 1,3-dicyclohexylcarbodiimide
DMAP = 4-(dimethylamino)-pyridine



Scheme II. Approach to the synthesis of the guaiacylglycerol-β-(FA-Ara) model.

Our previous procedure for the attachment of the α-L-arabinofuranosyl moiety to hydroxycinnamic acids was via preparation of the protected hydroxycinnamoyl chloride and subsequent coupling in pyridine. Due to the complexity of the protected free acid **6** (prepared in 7 steps from vanillin) and its susceptibility to degradation, a more facile esterification procedure needed to be developed. The use of 1,3-dicyclohexylcarbodiimide/4-(dimethylamino)pyridine (DCC/DMAP) in methylene chloride proved to be quite useful. The reactive imide forms the anhydride of **6** and subsequent coupling of the anhydride to the α-L-arabinofuranosyl unit is catalyzed by DMAP. These conditions are extremely mild and form the peracetylated model **7** in high yield. Efforts are underway to deacetylate this model in high yield without cleaving the hydroxycinnamoyl-arabinofuranoside ester linkage.

cleanly without preparation of the acid chloride with the use of 1,3-dicyclohexylcarbodiimide/4-(dimethylamino)pyridine (DCC/DMAP) in methylene chloride which gave the peracetylated model **7** in high yield (85%).

Discussion

Two of the major sites at which the crosslink can be predicted to be present are at the α- and β-positions of the arylpropane unit of the lignin monomer. Substitution at the α-site is due to the "opportunistic attack" of the phenolic hydroxyl of the hydroxycinnamic acid on the intermediate quinone methide, which was in turn produced during the lignification process. This reaction is not considered to be under enzymatic control whereas substitution at the β-position requires that hydroxycinnamic acids are directly involved in the lignification process. Thus the preparation and spectroscopic characterization of both **4** and **7** is an important step in understanding the fundamental aspects of cell-wall biosynthesis.

Conclusion

Procedures have been developed to prepare two important models which depict the proposed crosslink among lignin, hydroxycinnamic acids, and polysaccharides. Complete spectral characterization and comparison with synthetic DHP lignins as well as native tissue will provide important information concerning the regiochemistry of attachment of hydroxycinnamic acids to lignin and polysaccharides.

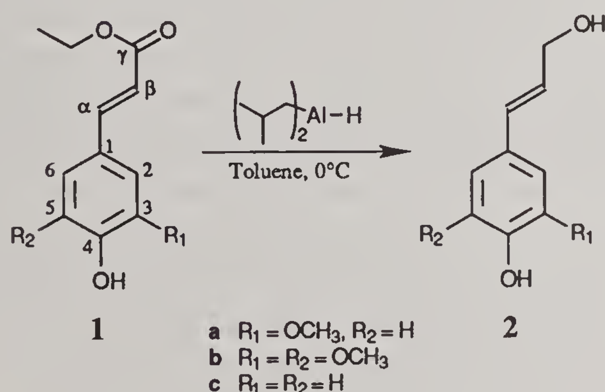
FACILE SYNTHESIS OF CONIFERYL, SINAPYL AND *p*-COUMARYL ALCOHOLS

S. QUIDEAU and J. RALPH

Introduction

Lignin-like dehydrogenation polymers (DHPs) can be made *in vitro*, using mushroom laccase or horseradish peroxidase preparations. Although synthetic dehydrogenative polymerization is a simplification of the lignification processes, it constitutes a unique tool to elucidate the lignin structural patterns and to study the possible chemical pathways followed during lignin biogenesis and degradation. However, such investigations have always been made difficult by the poor accessibility of the *p*-hydroxy-(E)-cinnamyl alcohols, namely coniferyl (**2a**), sinapyl (**2b**), and *p*-coumaryl (**2c**) alcohols.

In the past, lithium aluminum hydride reduction of ethyl ferulate (**1a**) was the most commonly used synthetic route toward **2a**. Sodium bis(2-methoxyethyl)aluminum hydride was later used as reductant in order to obtain better 1,2-selectivity in the reduction of the conjugated ester **1a**. In both cases, varying amounts of saturated alcohol were observed due to competing 1,4- vs 1,2-attack by hydride. The "ate" complex generated from diisobutyl aluminum hydride and *n*-butyllithium was used to achieve the desired chemoselective reduction of **1a** in 64% yield. Over the years, different synthetic routes leading to *p*-hydroxycinnamyl alcohols have been reported, but all demand several steps and/or give only moderate overall yields.



In this summary, we report that simple Dibal-H reduction of ethyl ferulate (**1a**) rapidly and cleanly affords coniferyl alcohol (**2a**) in good yield and allows large scale preparation. The method works equally well for preparing sinapyl (**2b**) and *p*-coumaryl (**2c**) alcohols.

Materials and Methods

NMR spectra were run in acetone-d_6 on a Bruker AMX-360 instrument with the central solvent signal used as internal reference (^1H, 2.04 ppm; ^{13}C, 29.8 ppm). Unambiguous assignments were obtained from proton-detected C-H chemical shift correlation spectra run with Bruker's invbtp pulse program.

Ethyl ferulate (**1a**) in toluene, under nitrogen, was cooled in an ice-water bath and diisobutyl aluminum hydride (4.2 eq) in toluene was slowly added . After addition was complete, stirring was continued for 1 h. The reaction mixture was then carefully quenched with ethanol. The solvents were partially removed *in vacuo* at 40°C. The residue was diluted in water and extensively extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and evaporated to dryness to give coniferyl alcohol (**2a**) as a white/pale yellow solid. Crystallization from dichloromethane/ petroleum ether afforded **2a** as colorless plates in 77% yield (m.p.77.9-78.6°C).

Discussion

Diisobutylaluminium hydride (Dibal-H) is well known as one the most versatile reducing agents used in organic synthesis because of its ability to achieve stereo- and chemoselective reductions, particularly in the case of unsaturated carbonyl compounds. Thus, **2a-c** were obtained from their corresponding ethyl cinnamate derivatives **1a-c** via Dibal-H reduction in toluene at 0°C, in 77%, 70% and 92 % yield, respectively. We have thus concluded that the

use of the “ate” complex from Dibal-H and *n*-BuLi is unnecessary, since it affords no improvement in achieving 1,2-selectivity in these reductions. The alcohols were characterized by NMR spectroscopy and the ^{13}C-NMR chemical shifts are given in Table 1. This simple reduction method allows a convenient preparation of labelled *p*-hydroxycinnamyl alcohols, since the ethyl cinnamate derivatives **1a-c** can be readily prepared with ^{13}C (or ^{14}C) labelling at any side-chain position. Such specifically labelled alcohols are of key importance in studies of lignin biosynthesis and biodegradation.

Summary

Coniferyl, sinapyl and *p*-coumaryl alcohols are rapidly and cleanly prepared by selective 1,2-reduction of the corresponding cinnamate esters using diisobutylaluminium hydride (Dibal-H) as the reducing agent in toluene.

Table 1. ^{13}C-NMR shifts of *p*-hydroxy-(E)-cinnamyl alcohols (solvent: d_6-acetone).

	α	β	γ	OCH_3	1	2	3	4	5	6
2a	130.4	127.9	63.4	56.1	130.1	109.9	148.4	147.1	115.7	120.5
2b	130.6	128.3	63.3	56.5	129.0	104.6	148.7	136.5	148.7	104.6
2c	130.1	127.7	63.4	-	129.7	128.3	116.1	157.7	116.1	128.3

SYNTHESIS OF ^{13}C-LABELED FA-ARA AND LIGNIN DEHYDROGENATION POLYMERS

J. RALPH, R.F. HELM, S. QUIDEAU and R.D. HATFIELD

Introduction

FA-Ara (methyl 5-*O*-trans-feruloyl-α-L-arabinofuranoside) was previously synthesized (see the 1991 Research Summaries) as a model for ferulic acid on arabinoxylans. From this model, we have been able to determine NMR parameters, examine the reactivity and stability of feruloyl esters, and assay feruloyl esterase activity in a variety of samples. In order to unambiguously determine how this feruloyl ester is attaching to lignin in plants, however, we sought to prepare strategically labeled ferulic acid, its arabinosyl ester, and a co-polymer DHP of this arabinosyl ester with coniferyl alcohol - the latter polymer should mimic the complex lignin/phenolic acid polymer in grasses.

Lignin is formed from *p*-hydroxycinnamyl alcohol monomers, primarily coniferyl alcohol and sinapyl alcohol, by an enzyme-initiated dehydrogenative polymerization. Resonance-stabilized phenoxy radicals are produced from monomers and from the evolving lignin macromolecules and these couple in a variety of ways to build up the enigmatic polymer. Intermediate quinone methides, which are produced from radical coupling reactions involving the β-carbon, typically undergo nucleophilic attack at the α-position. Water would be the predominant nucleophile present in the plant cell wall and would afford an a-hydroxyl group on β-ether structures. Free acids and alcohols could also attack the α-position of the quinone methides leading to α-esters

and α-ethers. In the case of feruloyl esters, the free phenol can trap quinone methides or become directly involved in the free radical polymerization process.

It is generally thought that attack on the quinone methide is the mechanism by which feruloyl esters become bound to lignin. This is a reasonable and predictable occurrence, the validity of which can be demonstrated in model systems. It has a number of frequently overlooked shortcomings however. Firstly, the feruloyl ester has to compete for the quinone methide with other nucleophiles including other phenols (from cinnamyl alcohol monomers and from the growing lignin polymer itself), acids present in other cell wall components (e.g uronic acids), and water. In isolated lignins, at least 90% of the β-ether quinone methides formed result in α-hydroxy structures indicating that water is the primary addition product. In cases where acids are in competition with phenols, esters are produced almost exclusively. A more philosophical question arises when feruloyl esters and lignification are considered in relation to the development of the plant cell wall. Feruloylated polysaccharides, typically arabinoxylans, are theorized to cross-link to lignin in order to impart various properties to the plant cell wall. Thus it seems rather unlikely that the method of cross-link formation would involve an uncontrollable reaction, especially when considering the spatial problem of placing a quinone methide sufficiently close to the feruloylated polysaccharide to produce the α-ether. Finally, although some peroxidase specificity has been demonstrated, it seems unlikely that these phenolic feruloyl esters would be available in the matrix for addition to quinone methides and yet not be amenable to H-abstraction and the radical coupling process. Consequently, we suspect that feruloyl ethers are also incorporated into the lignin structure through co-polymerization with lignin monomers. An implication of this hypothesis is that feruloyl ethers could become involved in a variety of structures, many of which would not solvolytically cleave to the parent ferulic acid monomers by present analytical methods (acidolysis, thioacidolysis, or high temperature base treatment).

Reactions which follow the initial radical-production step are independent of enzymatic control, and the lignification process can be effectively mimicked *in vivo* with the use of purified enzymes and hydrogen peroxide, providing synthetic dehydrogenation polymer (DHP) lignins. Incorporation of a strategically ^{13}C-labelled feruloyl ester of methyl-α-L-arabinofuranoside into a coniferyl alcohol DHP was seen as an effective way to determine the extent and detailed attachment regio-chemistry of incorporation of feruloyl esters into lignin polymers.

Methods

Triethyl phosphonoacetate is available, with labeling at the 1- or 2-position, from commercial sources. Reaction of the 1-^{13}C-labeled material with protected vanillin and subsequent removal of the phenolic protecting group and saponification of the ester produced the labeled ferulic acid in yields of about 75% (from vanillin). Subsequent chlorination and condensation with methyl 2,3-di-*O*-acetyl-α-L-arabinofuranoside **7**, generated the labeled FA-Ara in ca. 64% yield (Figure 1). The labeled FA-Ara was incorporated into lignin dehydrogenation polymers via a free radical co-polymerization as shown in Figure 2.

Discussion

The synthetic scheme to produce FA-Ara has been significantly improved with regard to the number of isolation and purification steps by exploiting multi-step one-pot reactions. This has led to an efficient synthesis that is ideal for making labeled substrates. The reason for choosing to label ferulic acid at the γ- or 9-position is because the ^{13}C NMR carbonyl resonance of this position is sensitive to ester cleavage (and hence provides informative NMR assays of ester cleaving reactions), and to substitution at the β- or 8-position that may occur (see accompanying article) when feruloyl esters are co-polymerized into lignins.

Co-polymerization of the feruloyl ester of methyl-α-L-arabinofuranoside into the traditional coniferyl alcohol DHP polymer (Figure 2) did occur, as evidenced by the non-extractability of any monomeric products containing the ^{13}C label. As will be seen in the accompanying report, NMR analysis showed not only was it intimately incorporated, but revealed a range of (predictable) structural types, some of which bound the ferulic acid in linkages from which it would not subsequently be hydrolysable to give ferulic acid.

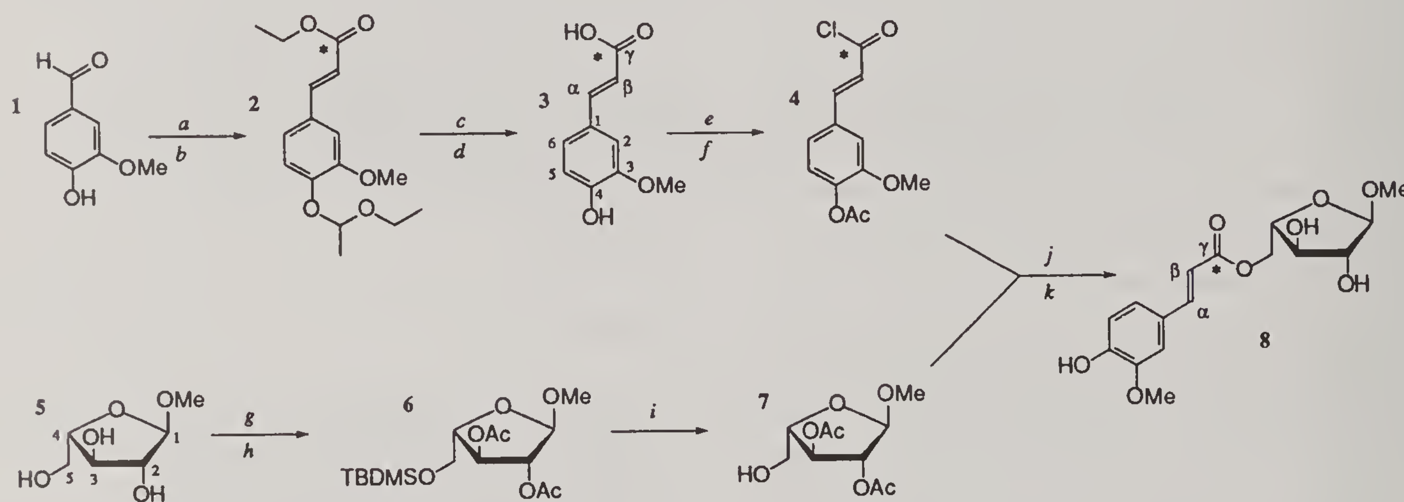


Figure 1. Synthetic scheme for preparation of 9-labeled FA-Ara.

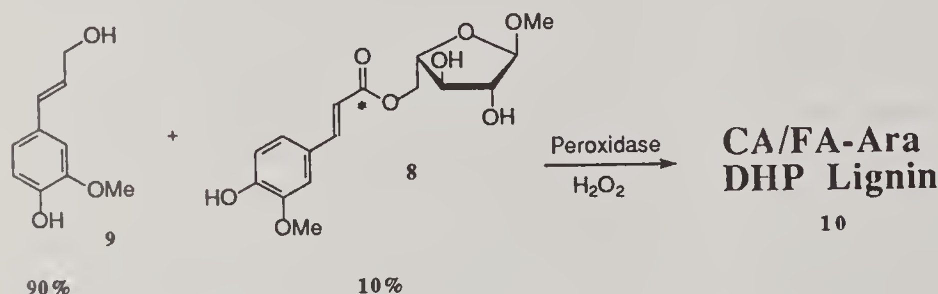


Figure 2. Scheme for incorporation of 9-labeled FA-Ara into a coniferyl alcohol DHP.

NMR DETERMINATION OF THE REGIOCHEMISTRY OF INCORPORATION OF FERULOYL ESTERS INTO CONIFERYL ALCOHOL DEHYDROGENATION POLYMERS

J. RALPH, R.F. HELM, S. QUIDEAU and R.D. HATFIELD

Introduction

Ferulic acid (4-hydroxy-3-methoxy-*trans*-cinnamic acid) in grasses is implicated in cross-linking cell wall carbohydrates to lignins. Whereas its attachment to carbohydrates, as esters, is relatively well defined, the regiochemistry of its attachment to lignin is not well understood. The determination of the nature and scope of lignin-hydroxycinnamic acid-polysaccharide interactions in plant cell walls is critical towards our understanding of cell wall biogenesis and degradation. Feruloyl esters can become 'opportunistically' involved in cross-linking by trapping lignin quinone methide intermediates, and/or be directly involved in the free-radical polymerization process. In the former process, simple α-etherified structures would result, whereas co-polymerization with lignin monomers would potentially result in a variety of structures, only some of which would be subsequently identifiable, by present solvolytic methods, as arising from ferulic acid.

Direct elucidation of ferulic acid/lignin connectivity via NMR spectroscopy (while the most unambiguous and structurally revealing method) is a challenge firstly because of sensitivity restrictions even with modern inverse-detection methodologies and, secondly, because of the large number of potential structures that might be important. Our preliminary efforts have therefore been directed toward model approaches to unambiguously obtain the required chemical shift and coupling constant data for compounds of interest and to optimize correlation experiments aimed at detecting these structures in plant cell wall isolates. In this study, after determining that feruloyl esters could be co-polymerized into the lignin structure via radical process, it was critical to elucidate the reaction regiochemistry (i.e. where they are attached and in what kinds of structures).

Method

Long-range C-H correlation NMR experiments are particularly valuable for establishing connectivity. Figure 1 shows a small subsection, incorporating only the carbonyl carbon region, of a 48 hr ^{1}H-detected long-range 2D C-H correlation spectrum of a 9-^{13}C-labelled FA-Ara/coniferyl alcohol DHP (described in an accompanying report), and the corresponding region of several dimers (Figure 2) synthesized for proof of the structural types assigned (complete detail not shown in this report).

Results and Discussion

It is evident from the ^{13}C spectrum, shown as the vertical projection in Figure 1, that the single carbonyl in the precursor monomer FA-Ara has generated a variety of carbonyl moieties in the polymer that are both saturated and unsaturated (groupings labeled A-D). The long-range correlations from the labeled carbonyl carbon to protons 2 or 3 bonds away are particularly diagnostic and provide evidence for β-O-4, β-5, 4-O-β and β-β products of the types shown in Figure 2.

It is important to note that, of the product types depicted in Figure 2, only the 4-O-β

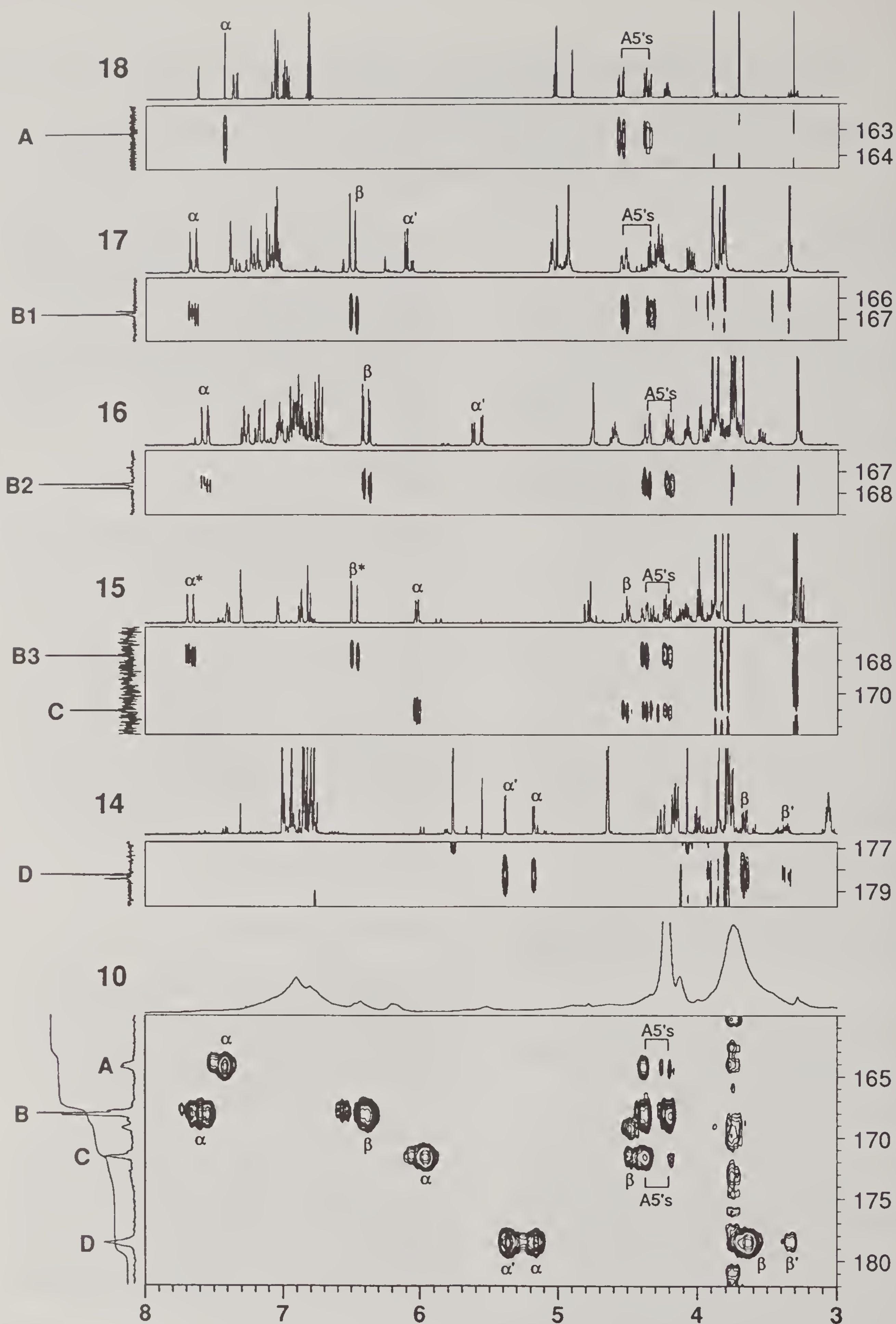


*Figure 1. Partial Long-range ^{1}H-detected C-H correlation spectra of FA-Ara DHP **10**, and model compounds **14-18** (used for assignment authentication). Important 2- and 3-bond correlated proton peaks to the carbonyls shown, are labeled.*

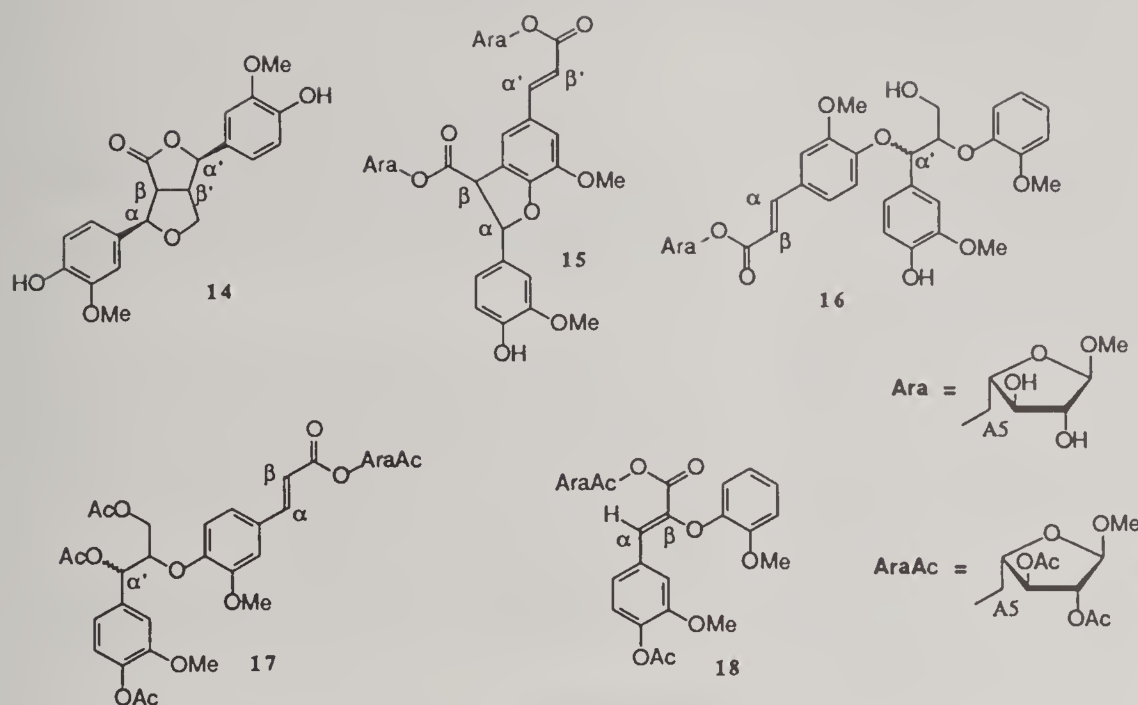


*Figure 2. Compounds synthesized for authentication of structural assignments in the FA-Ara DHP **10**.*

product **17**, the 4-O-α product **16**, and possibly the β-O-4 product **18** could regenerate ferulic acid (or logical derivatives) upon acidolysis, thioacidolysis, or high temperature alkaline solvolysis. Examination of the integrals on the carbon plot in Figure 1 shows that cluster A represents 9% (and may give ferulic acid products) and cluster B 37%. While the major peaks of cluster B probably arise from phenolic α- or β-ethers that are not further condensed, there are structures in this region that are condensed (structures B3-B6, or the condensed structures indicated by the dashed bonds in B1 and B2). We estimated from these spectra that a maximum of 40% of the products from this reaction would be extractable. Hans Jung has determined the extractability on this DHP and recovers only 8% ferulic acid.

Conclusion

Feruloyl esters, if present in the lignifying matrix, are clearly capable of entering into the free-radical polymerization process generally associated with lignification. Providing peroxidases are sufficiently non-specific, H-abstraction from these free-phenolic feruloyl esters and co-polymerization into the lignin polymer seems inevitable, and must at least compete with simple 'opportunistic' trapping of intermediate quinone methides to generate α-ethers. Among the implications are that ferulic acid is very likely involved in structures from which it cannot be released and quantitated by current solvolytic methods. Consequently, the quantity and importance of ferulic acid in non-woody plant cell wall complexes is probably being underestimated.

SYNTHESIS OF DIMERIC FERULIC ACID-LIGNIN MODEL COMPOUNDS VIA SILVER (I) OXIDE DEHYDROGENATION

S. QUIDEAU, J. RALPH and R.F. HELM

Introduction

Although *p*-hydroxycinnamic acids are minor components in the plant cell wall, they appear to have a significant role in regulating wall formation and in decreasing forage digestibility by rumen microorganisms. Being difunctional, these phenolic acids are potentially able to cross-link lignin and hemicelluloses, creating an extended three-dimensional gel structure which would affect cell wall polysaccharide degradation. A precise understanding of the chemical relationships between phenolic acids and the cell wall matrix is essential for the development of efficient chemical and/or biological treatments increasing forage digestibility.

In grasses, although ferulic acid was found to be selectively esterified to arabinoxylans via the primary hydroxyl of the arabinose residue, its attachment to lignin is poorly understood. In our efforts to elucidate the regiochemistry of *p*-hydroxycinnamic acids on forage lignins, methyl 5-*O*-feruloyl-α-L-arabinofuranoside (FA-Ara), selectively ^{13}C-labelled at the carbonyl position, was incorporated into a dehydrogenation polymer from coniferyl alcohol. A proton-detected long-range ^{1}H-^{13}C chemical shift correlation spectrum of the resulting DHP revealed critical connectivities that allowed us to propose cross-linking structures between lignin and arabinoxylans. Model compounds were needed for establishing chemical shift assignments and structural identification. In addition to the previously suggested α- and β-ether structural units, phenylcoumaran (β-5 coupling) and epoxylactone (β-β coupling) structures were also identified. The synthesis of their corresponding model compounds, **1** and **2** respectively, was accomplished via radical coupling using Ag_2O as a one-electron oxidant.

Methods

Ag_2O on celite (1.2 eq) was added to FA-Ara dissolved in acetone, and the mixture was stirred in the dark at ambient temperature for 72 h. Preparative TLC (elution with 20:1-CH_2Cl_2:MeOH) of the oil obtained after filtration and evaporation afforded **1** in 55% yield.

A 1:1.5 mixture of ferulic acid:coniferyl alcohol was similarly reacted for 30 min and the resulting reddish oil was submitted to preparative TLC (same eluent as for **1**). The less polar fraction afforded a 1:1.5:2.5 mixture of pinoresinolide (**2**), dehydrodiferulic acid di-γ-lactone (**3**) and pinoresinol (**4**), respectively, in 10% yield.

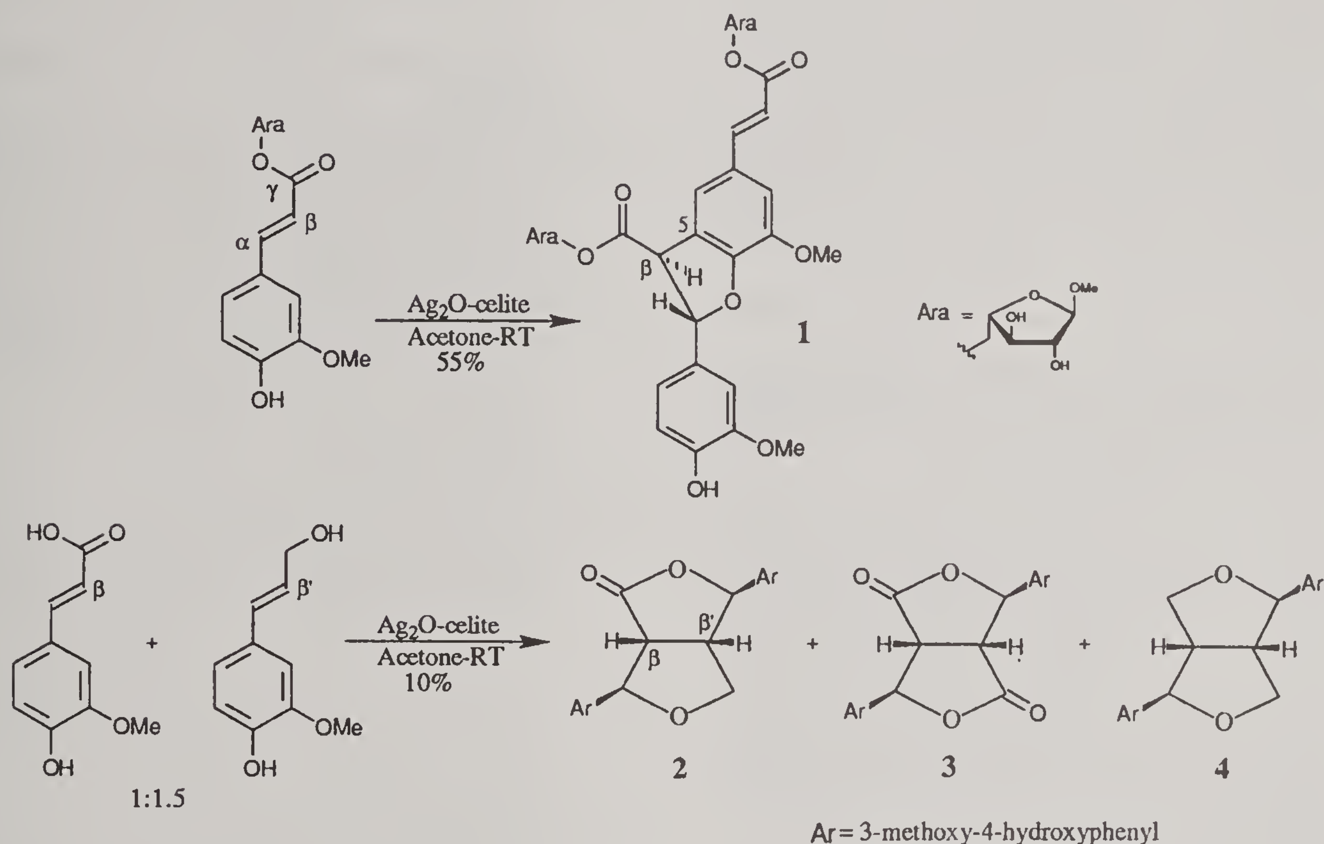
Discussion

The use of silver (I) oxide, in organic solvent, as a one-electron oxidant was proven to be an efficient reagent for the synthesis of condensed dimeric lignin-type model compounds, allowing duplication of the structures produced with the peroxidase-H_2O_2 aqueous system upon dehydrogenative polymerization. Of particular note is the formation of pinoresinolide units, which cannot be seen as cross-linking structures since the arabinosyl group has been cleaved, but proves the effective incorporation of feruloyl groups into coniferyl alcohol dehydrogenation polymers. If cell wall bound ferulic acid is directly involved in the peroxidase-catalyzed dehydrogenation, it seems likely that the β-5 and β-β coupled structures identified here would also result *in vivo*.

It is also noteworthy that the syntheses of phenylcoumarans via traditional synthetic routes are rather difficult and give only low overall yields. Oxidative coupling with Ag_2O, together with the now facile synthesis of coniferyl alcohol, should provide an attractive method for the preparation of such structures, as well as a first convenient step toward the synthesis of higher oligomeric lignin model compounds.

Conclusion

Silver (I) oxide dehydrogenation of FA-Ara and ferulic acid:coniferyl alcohol mixture was successfully applied to the synthesis of dehydrodiferuloylated arabinofuranoside (phenylcoumaran) (**1**) and pinoresinolide (**2**), respectively. These compounds model structures arising from the dehydrogenative co-polymerization of FA-Ara with coniferyl alcohol. The possibility *in vivo* of such condensed, non-hydrolyzable structures would imply a higher contribution and occurrence of ferulic acid in the cell wall than usually supposed.



INTERNATIONAL SYMPOSIUM ON FORAGE CELL WALL STRUCTURE AND DIGESTIBILITY

Organizational Committee
R.D HATFIELD, D.R. BUXTON, H.G. JUNG, D.R. MERTENS, J. RALPH and P.J. WEIMER

The International Symposium on Forage Cell Wall Structure and Digestibility was held at the Inn on the Park in Madison, Wisconsin on Oct. 7-10, 1991. This symposium brought together a group of people representing the diverse areas that make up the broad topic of forage cell wall utilization. A total of 165 people from 15 different countries attended the symposium. Approximately 25% of the people attending were from foreign countries. The first two of the three major goals developed by the organization committee were accomplished at the symposium. These goals were: 1) to bring together an interdisciplinary group with interests ranging from cell wall structure and function to cell wall utilization by ruminants; 2) to compile the current state of

knowledge and to focus attention on the future directions in the area of forage cell wall structure and utilization; 3) to publish the symposium proceedings as a benchmark reference on cell wall structure as it relates to digestibility. The proceedings will be available in the fall of 1992.

The format of the symposium was seven half day sessions with three presentations per session. Adequate time was provided after each talk and again after each session to encourage and maximize exchanges among the participants of the symposium. Several participants enriched the symposium with presentations of their own data during the poster sessions. A total of 23 posters were presented during the Monday and Tuesday evening sessions. Nearly all of the symposium participants and numerous guests attended the banquet on Wednesday evening and listened to the keynote address given by Dr. Gordon Marten.

A post symposium questionnaire was completed by 25% of the participants. Generally all comments were very favorable. All seven sessions received high marks (≥ 3.8 out of 5.0) with sessions III (Structure of forage cell walls) and IV (Cell wall matrix interactions and degradation) receiving the highest marks (4.3). Most participants found the symposium to meet or exceed their expectations and was well worth the time and expense. The most common comment was that they were impressed by the diversity of disciplines attending and the large amount of interaction that was fostered among them.

Speakers, session moderators, and other interested parties attended a post symposium workshop to develop a list of critical areas for research. The results of that meeting are listed below. Most critical information needed to improve forage cell-wall digestibility by ruminants:

1. Which changes in structural and conformational arrangements of cell-wall polysaccharides and lignin will lead to increased cell wall digestibility?
2. Does lignin have to be cross-linked to cell-wall polysaccharides to limit their digestibility?
3. Which factors within plant cells control lignification and how can these be modified to affect the amount and chemical composition of lignin?
4. Can microbial or animal genetic modifications increase the amount of energy available to animals from forage cell walls? If so, which modifications will be most effective?
5. Are numbers, types, and activities of rumen microorganisms limiting for forage cell-wall digestibility and how can these be adequately measured *in vivo*?
6. What are the rate limiting steps and necessary components for enzymatic hydrolyses of cell walls by rumen microorganisms and what methods can be used to identify these steps?
7. Is unavailability of forage cell-wall surface to rumen microorganisms a major deterrent to digestibility of cell walls?
8. Are lignified cell walls of forages digested only from the lumen side by rumen microorganisms?
9. What plant modifications will lead to increased particle size reduction in the rumen and how will these affect the amount of energy available to animals from forage cell walls?
10. How will changes in rate and extent of forage cell-wall digestion in the rumen affect the amount of energy available to animals?
11. Will improvement in forage cell-wall digestibility have negative associative affects on yield, vigor, and ability of forages to withstand stressful environments and pests?

QUICK-DRYING FORAGE MATS

R.G. KOEGEL, T.J. KRAUS, R.J. STRAUB and K.J. SHINNERS

Introduction

Mats made from alfalfa macerated at the time of mowing and placed on the stubble have been shown in earlier research to dry to a moisture content suitable for baling in less than 6 hours under favorable conditions. Furthermore, alfalfa so harvested proved to have more rapid and extensive dry matter digestibility (generally 10-15% increase) than conventionally harvested material.

Efforts during the past year have been to design and construct a "second generation" forage mat machine based on the crushing-impact macerator described in last year's report. In addition, another feeding trial was completed.

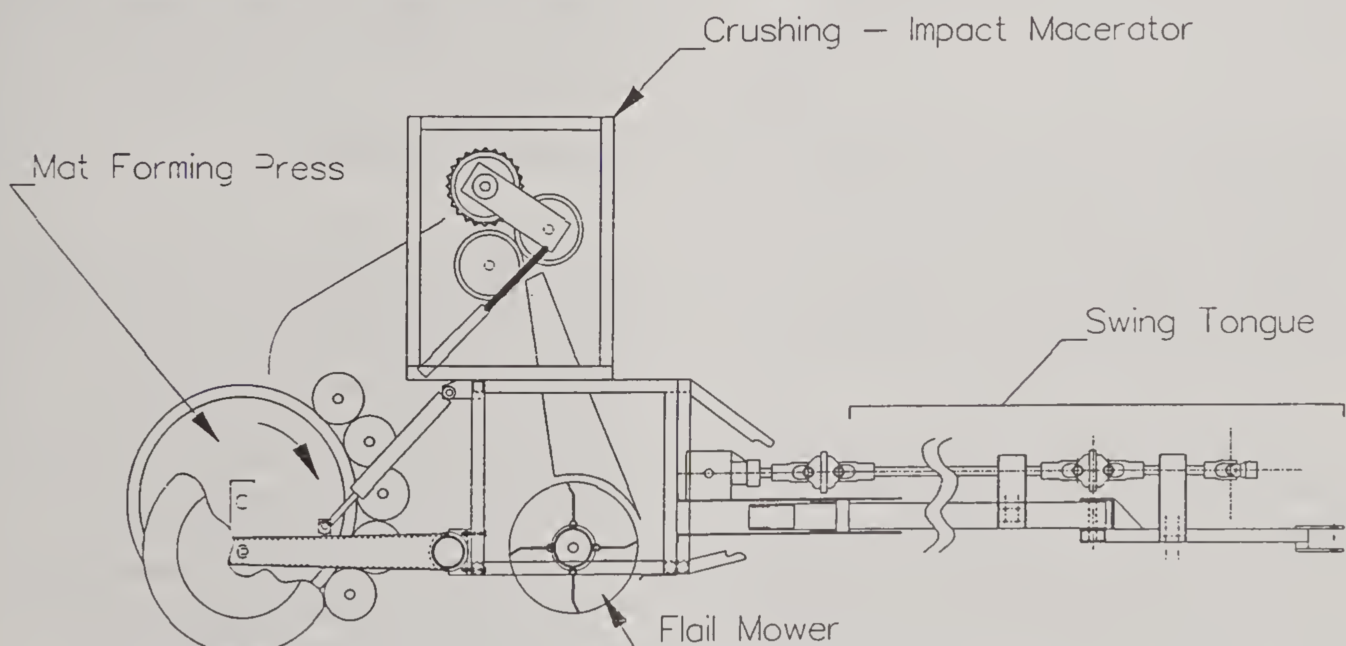
Materials and Methods

Design features of the pull-type machine (Fig. 1) which was constructed during the spring and summer of 1991 include: (1) a crushing-impact macerator, (2) a beltless mat-forming press consisting of a drum and multiple pressure rolls, (3) a swing tongue which allows the machine to be towed directly behind the tractor or offset to either side, and (4) a flail mower which delivers the forage to the macerator.

Results and Discussion

Since previously formed mats should not be run over by the tractor, the swing tongue provided the options of straddling the mat formed on the previous round or of running over the crop immediately prior to mowing it. As the technology is scaled up and mats become wider, straddling previously made mats becomes less practical.

The flail mower exhibited at least two advantages relative to other mower types: (1) it delivered the crop in a uniform flow to the macerator while eliminating the need for a reel or conveyor and (2) it was able to pick up any crop which was lying down.



As reported in 1990, the crushing-impact macerator performed acceptably while reducing (1) the number of moving parts, (2) the need for exact clearances, and (3) the specific energy requirement.

Since the mat-forming press was completed late in 1991, it could not be evaluated extensively. After initial trials, the surfaces of both the drum and the five pressure rolls were modified to improve mat release. Further evaluation of the press, and of the machine as a whole, will resume in May 1992.

An additional feeding trial comparing both hay and silage made from mats with conventional harvested control material was completed. Results of this trial are reported elsewhere in this publication by D. R. Mertens.

Conclusions

Progress has been made on simplifying the equipment, increasing the harvesting rate, and reducing the energy requirement for the mat harvesting process. Information on the feeding value and feeding strategies for macerated forage products continues to be developed.

In addition to the USDFRC machine described above, at least seven other prototype machines are being evaluated elsewhere. With the exception of one Canadian machine, all of these are in Western Europe.

ENERGY REDUCTION IN FORAGE HARVESTING

R.G. KOEGEL, K.J. SHINNERS and T.A. EVERTS

Introduction

The power requirement of forage harvesting is frequently a limiting factor which determines the rate at which harvesting can be accomplished. Since timeliness of harvest reduces risk of weather damage, a lower power requirement can lead to improved forage. In addition, use of a smaller power unit reduces both capital and operating costs.

Approximately 40% of power required by a forage harvester is used for the blower which functions at approximately 5% efficiency. Past efforts have centered on elimination of the blower by means of inverting the cutterhead and making use of kinetic energy imparted to the forage to convey it to the trailed wagon.

Energy required by the cutterhead is roughly inversely proportional to the theoretical length of cut. Because forage enters the cutterhead in a disoriented fashion, particle length varies widely and results in a certain fraction of long particles. Because these long particles adversely affect the functioning of unloaders in tower silos, farmers tend to set their harvesters for a shorter theoretical length of cut than might otherwise be desirable in an effort to minimize long particles. This results in increased energy consumption. Efforts this year were on a mechanism to orient the forage entering the cutterhead to improve the uniformity of the particle size and thereby allow a longer theoretical length of cut to be used.

Materials and Methods

A mechanism to comb and properly orient legume and grass crops between the forage harvester cutterhead and its feed rolls was designed and integrated into an upward cutting cut-and-throw forage harvester. Extensive field tests utilizing different combing ratios were conducted. Combing ratio is the ratio of the tip speed of the comber to the tip speed of the feed rolls. Performance of the mechanism was quantified by particle size analysis (percent long fibers, geometric mean particle length,

geometric standard deviation) and overall machine energy requirements.

Results and Discussion

Combing the crop before the cutterhead reduced the geometric mean particle length by 28%, the geometric standard deviation by 15% and reduced the percent of long particles by 51% when harvesting third crop alfalfa compared to operating the experimental forage harvester with no combing. However, the experimental forage harvester, when operated at the highest combing ratio (3:1), provided a length-of-cut performance no better than a control forage harvester. Combing ratios of greater than 3:1 were not considered advantageous. Energy requirements for combing were less than 0.2 hph/t. Future work will include a redesign of the combing mechanism and feed rolls.

Conclusions

Forage orientation devices can improve the uniformity of particle length. This, in turn, would allow the use of a longer theoretical length of cut which reduces the specific energy requirements. Representatives of agricultural machine manufacturers have voiced enthusiasm for this approach.

HARVEST AND STORAGE LOSSES WITH ALTERNATIVE FORAGE HARVESTING METHODS

C.A. ROTZ, L.R. BORTON and J.R. BLACK

Introduction

Considerable loss occurs during the harvest and storage of alfalfa. This loss is related to the crop moisture content at harvest as well as the field curing time required to attain this moisture. About 30 years ago, C.R. Hoglund, an agricultural economist at Michigan State University, did an extensive literature review on dry matter losses in forage harvest and storage. With the information gathered, a graph was created to illustrate these losses from direct-cut harvest to very dry field-cured hay. This graph is widely used in teaching and extension programs to show the magnitude of forage losses.

Changes have occurred during the past 30 years which may enable a better representation of these losses. Major changes include: more qualitative and quantitative knowledge of forage losses, new harvest technology, and a greater awareness of the importance of forage quality. The value of quality changes may be greater than the value of yield losses. The dairy forage system model (DAFOSYM) integrates all of these changes to give more accurate prediction of loss as influenced by crop, machinery and environmental conditions. This comprehensive model incorporates the latest information on losses and quality changes during alfalfa harvest and storage to predict their impact on animal performance. A study was undertaken to describe forage losses with DAFOSYM.

Materials and Methods

DAFOSYM was used to create graphs with a similar format as the original Hoglund graph that represent 1) the loss of forage dry matter and 2) the loss of total feed value based upon both dry matter and nutrient constituents. Losses and their value were determined for a representative farm near East Lansing, Michigan. A farm that included 61 hectares each of alfalfa and corn was simulated for 26 years of historical weather. For hay harvest, a three-cutting system was used with alfalfa harvested in late May, mid July and late August. A four-cutting system was used for alfalfa silage harvest with similar harvest dates plus a fourth cutting in mid October. In hay systems, alfalfa was dried in wide swaths

and raked prior to baling. For silage systems, narrow swaths were used with no manipulation prior to chopping. Direct-cut and high-moisture silages were stored in bunker silos, and formic acid was applied to direct-cut silage to enhance preservation. Low-moisture silage (haylage) was stored in sealed silos.

Analyses were done for both dry matter loss and loss of feed value. Average dry matter loss was determined by simulating the same farm both with and without rainfall and other loss parameters set to zero. Loss was expressed as a percent of the crop available at the time of mowing. Loss of feed value was the difference in the net return above feed costs with and without dry matter losses included in the model. Percent loss was the loss of feed value divided by the market value of the crop at mowing. Initial market value was determined by simulating the farm without all losses included.

Results and Discussion

Average dry matter losses predicted by DAFOSYM for various harvest systems are illustrated in Figure 1. When compared to the original Hoglund graph, a more abrupt change in loss occurs between harvest systems. This should be expected since major changes in harvest and storage technology cause loss differences that cannot be properly illustrated by a smooth, continuous curve. Harvest and storage losses for dry hay systems are similar to those presented 30 years ago. Loss in harvesting field-cured hay is slightly greater and high-moisture hay is slightly less than the previous data. Dry matter loss in silage storage was similar to Hoglund's data, but our model predicted slightly higher harvest losses in wilted silage.

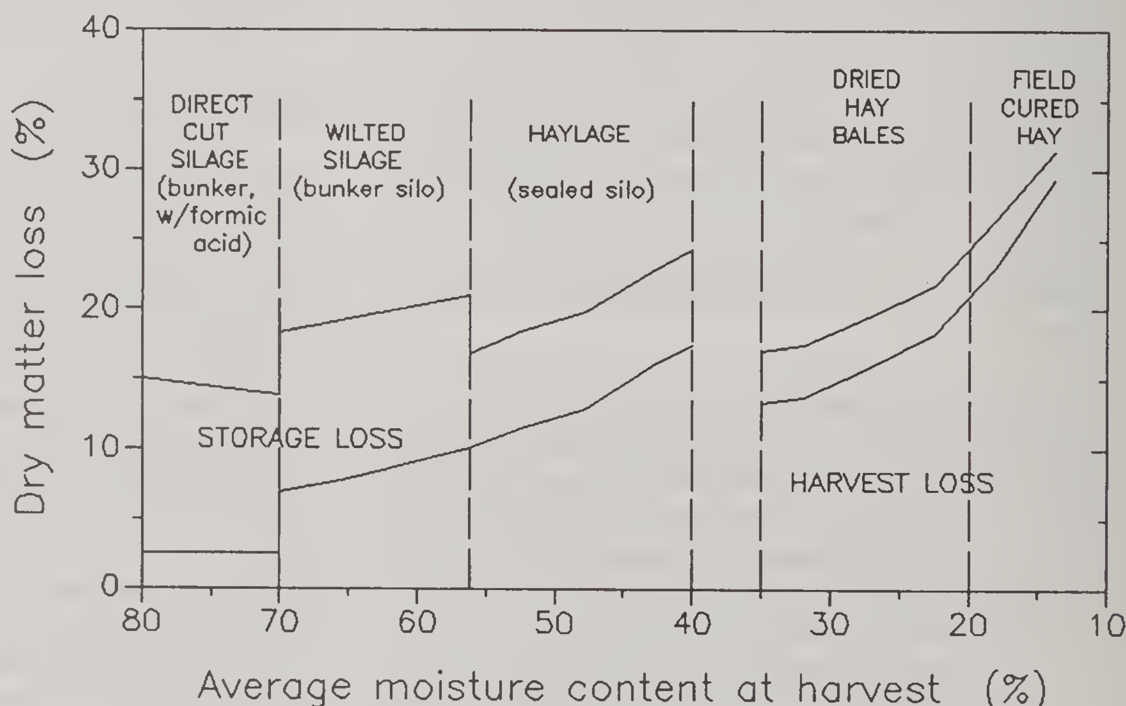


Figure 1. Dry matter losses for alfalfa harvested at various moisture levels averaged for 26 years at East Lansing, Michigan weather.

For direct-cut silage, formic acid was applied at a rate of 1% dry matter to help stabilize fermentation. This treatment reduced storage loss.

The percentage loss in feed value during harvest and storage was similar to the loss of dry matter when the alfalfa was fed to a moderate producing (18,000 lb) dairy herd. Lost value included lost income due to decreased sales of surplus corn and alfalfa and increased purchases of soybean meal to replace lost and damaged protein. When fed to a high producing (>20,000 lb) herd where milk production was limited by forage quality, the loss in feed value was much greater (40-55%). This loss included lost income due to decreased sales of milk as well as the increased feed costs. Loss of feed value was similar across systems except for direct-cut silage.

Conclusion

Total dry matter loss in the harvest and storage of alfalfa is between 15 and 30% for most systems, but the loss in feed value is 40 to 55% when alfalfa is fed to a high-producing dairy herd. The graphs of forage losses provide educators more current information on typical losses in the northern U.S.

A COMPARISON OF HAY MAKING SYSTEMS ON VIRGINIA FARMS

C.A. ROTZ

Introduction

Hay growers are presented with a variety of products and strategies promoted to make better hay. The many choices tend to create confusion. Given the technology available today, what is the best way to make hay? Which products and strategies provide economic return and which do not? A study was undertaken to answer these questions for Viriginia hay growers. One method of comparing various technologies is to conduct a large experiment over several years where the technology is compared in side-by-side plots. This experiment would be very costly if not impossible. Another approach is to perform the comparison with model farms using computer simulation. DAFOSYM provides an excellent tool for making side-by-side comparisons in a short time and at a relatively low cost.

Materials and Methods

DAFOSYM was used to simulate two representative farms for 25 years of Roanoke, Virginia weather conditions. The first farm included 200 acres of alfalfa and corn with 75 acres of alfalfa harvested for commercial hay. A four cutting harvest system was used with harvests beginning within 5 days of May 12, June 15, July 21 and August 26. The average post harvest yield was 4.1 ton dry matter (DM)/acre. Harvest equipment included a 12 foot mower-conditioner, a tandem rake, a medium-sized baler, three bale wagons and three tractors of 47 to 87 hp. All hay was sold at a price set proportional to its relative feed value. Prices ranged from $60 to $120/ton DM with an average price of $90/ton DM.

The second farm was modeled as a typical Virginia dairy farm with 80 cows producing 16000 lb/cow. Fifty acres of alfalfa were used to supply about 25% of the herd's forage needs. A four cutting harvest system was again used where first and fourth were chopped and stored as wilted silage and second and third were harvested as dry hay. Harvest equipment was similar to that used on the hay farm but included a small baler, a medium-sized forage harvester and a 108 hp tractor. The economic return for the farm was the difference between milk income and the total cost of feed production and feed supplementation for the herd. The major portion of feed came from 120 acres of corn harvested as silage and grain.

Results and Discussion

Technologies evaluated included rotary disk mowers, mechanical and chemical conditioning, tedding, use of round bales, use of preservatives on moist hay, barn drying of moist hay and a new process for rapid drying of macerated hay in mats (Table 1). The most promising technologies were mechanical conditioning, round baling with hay stored under cover and the new mat process. Disk mowers, chemical conditioning, and barn drying of high-moisture hay increased the amount and quality of hay produced, but their economic benefit was small. Tedding, round bales stored outside and hay preservatives on moist hay were uneconomical and they reduced the yield and quality of hay produced. Comparisons of the benefits and economics of alternative hay making technologies provide hay producers with new, useful information for managing their farms.

Table 2. Effects of several harvest alternatives on field-curing time, amount of hay produced, average hay quality, production costs and net return on two types of farms near Roanoke, Virginia.

Harvest alternative	Curing time		Amount of DM		Quality		Increased cost	Increased return
	HQH* (day)	MQH (day)	HQH (ton)	MQH (ton)	CP (%)	NDF (%)	($/acre)	($/acre)
Commercial hay farm								
Base system	2.8	5.6	143	164	20.7	44.5	---	---
Rotary disk mower	2.9	5.9	155	154	20.7	44.2	9	-2
No conditioning	3.2	6.9	98	140	20.7	45.6	-18	-75
Chemical conditioning	2.1	5.4	168	146	20.6	43.8	21	-2
Tedding after rain	2.7	5.3	138	149	20.4	44.6	12	-38
Tedding all hay	2.6	5.0	122	148	20.3	44.6	16	-62
No raking	3.0	6.2	138	171	20.7	44.8	- 9	9
Round baling, stored inside	2.7	5.9	136	167	20.8	44.6	-25	19
Round baling, stored outside	2.7	5.9	122	149	20.3	49.8	-43	-54
Moist hay with preservative	2.2	5.1	166	148	21.4	45.1	67	-66
Barn drying w/ambient air	2.2	5.1	171	152	21.0	43.8	39	- 8
Mat process	1.0	2.0	240	120	20.4	41.4	84	25
Dairy farm								
Base system	2.4	5.8	64	47	21.0	43.0	---	---
Rotary disk mower	2.5	5.8	66	46	20.9	42.8	14	-10
No conditioning	2.8	5.9	49	49	21.0	43.9	-26	- 8
Chemical conditioning	1.9	5.5	72	42	20.9	42.4	12	- 6
Tedding after rain	2.2	5.0	61	41	20.8	42.9	13	-31
Tedding all hay	2.1	4.8	58	40	20.7	42.7	16	-40
No raking	2.4	5.8	60	48	21.0	43.3	-43	25
Round baling, stored inside	2.4	5.9	62	51	21.1	43.1	-13	18
Round baling, stored outside	2.4	5.8	54	43	20.5	48.3	-52	2
Moist hay with preservative	2.1	5.7	69	44	21.3	43.1	18	-16
Barn drying w/ambient air	1.9	5.1	72	43	21.3	42.2	24	-10
Mat process	0.9	1.8	90	34	20.6	41.0	1	99

*HQH = high quality hay (< 41% NDF); MQH = moderate quality hay (> 41% NDF); CP = crude protein; NDF = neutral detergent fiber.

ECONOMIC POTENTIAL OF PRESERVING HIGH-MOISTURE HAY

C.A. ROTZ, D.R. BUCKMASTER and L.R. BORTON

Introduction

Baling high-moisture hay with an effective preservative can reduce rain damage and baling losses, reduce or eliminate storage losses and may maintain or improve forage quality. Many hay preservatives have been used, yet none have met wide acceptance. Proposed preservatives include anhydrous ammonia, propionic and other organic acids, urea, sodium diacetate and bacterial inoculants. Propionic acid, organic acid mixtures and ammonia have proven effective while others have not.

An issue that has not been fully addressed is the economic value of preserving high-moisture hay. The risk associated with weather effects on hay making must be considered in determining the economics of baling and preserving high-moisture hay. DAFOSYM is a model of forage production and utilization that simulates the timing of field operations, drying, storage changes and effects of nutritive value on economic returns. The objective of this project was to use DAFOSYM to determine the economic value of a wide range of potential treatments with various strategies of use for hay preservation.

Materials and Methods

Breakeven costs for preserving high-moisture hay were determined for three synthesized, representative farms: 1) a commercial hay farm, 2) a small dairy farm (50 cows) with all alfalfa harvested as hay, and 3) a larger dairy farm (100 cows) with alfalfa harvested as both hay and silage. Each farm was simulated for 26 years of historical weather for East Lansing, Michigan. Simulations with hay baled wet (up to 28% moisture) were compared to those for dry hay (up to 20% moisture). Increased return for the farm was the increased income from feed and milk sales minus any change in production costs. The increased return was divided by the amount of hay treated to obtain a breakeven cost. The cost of the treatment must be less than the breakeven cost to realize an increase in farm profit.

The economics of hay preservatives were determined for three strategies of use and three levels of effectiveness. The three strategies of use were defined as limited, moderate and heavy. Under limited use, limited amounts of treated hay were baled when the probability was high for avoiding rain damage. Moderate use attempted baling all hay as high-moisture hay. Some dried enough for stable storage without treatment (below 20% moisture) while waiting for other plots to be baled and was not treated with a preservative. For heavy use, all hay was treated regardless of moisture content.

The three levels of effectiveness of hay preservatives were defined as normal, excellent and ideal. Normal effectiveness was modeled similar to preservation with propionic acid (60% reduction in heating with a storage loss the same as that in untreated high-moisture hay). Hay treated with an excellent preservative was assumed to have the same heating, dry matter (DM) loss and quality change during storage as dry hay (18% moisture). For an ideal preservative, all loss and quality change during storage were eliminated.

Results and Discussion

Hay treatments with normal effectiveness (similar to propionic acid) must be applied at a total cost of less than \$8/t DM in order to provide economic return with limited use (Figure 1). When treating most or all hay, the cost must be less than \$4/t DM. If this treatment is used only when it prevents rain damage to the crop (considering perfect knowledge of future weather), the cost can be as high as \$24/t DM with a net economic

gain. The cost of propionic acid treatment is more than double the breakeven cost of normal preservation with limited use. Hay treatments with excellent effectiveness have a breakeven cost of about $9/t DM when used on most hay and up to $15/t DM for limited use to reduce the probability of rain damage (Figure 1). The higher breakeven cost under limited use implies that field-cured hay is more economical when good weather is assured. Heavy use lowers the breakeven cost since no benefit comes from the treatment of dry hay. Hay treatments with ideal effectiveness (eliminate all storage loss) provide breakeven costs of $11 to $16/t DM when applied to all hay (Figure 1). When the treatment is used selectively, the cost can be as high as $21/t DM and still result in a net economic gain.

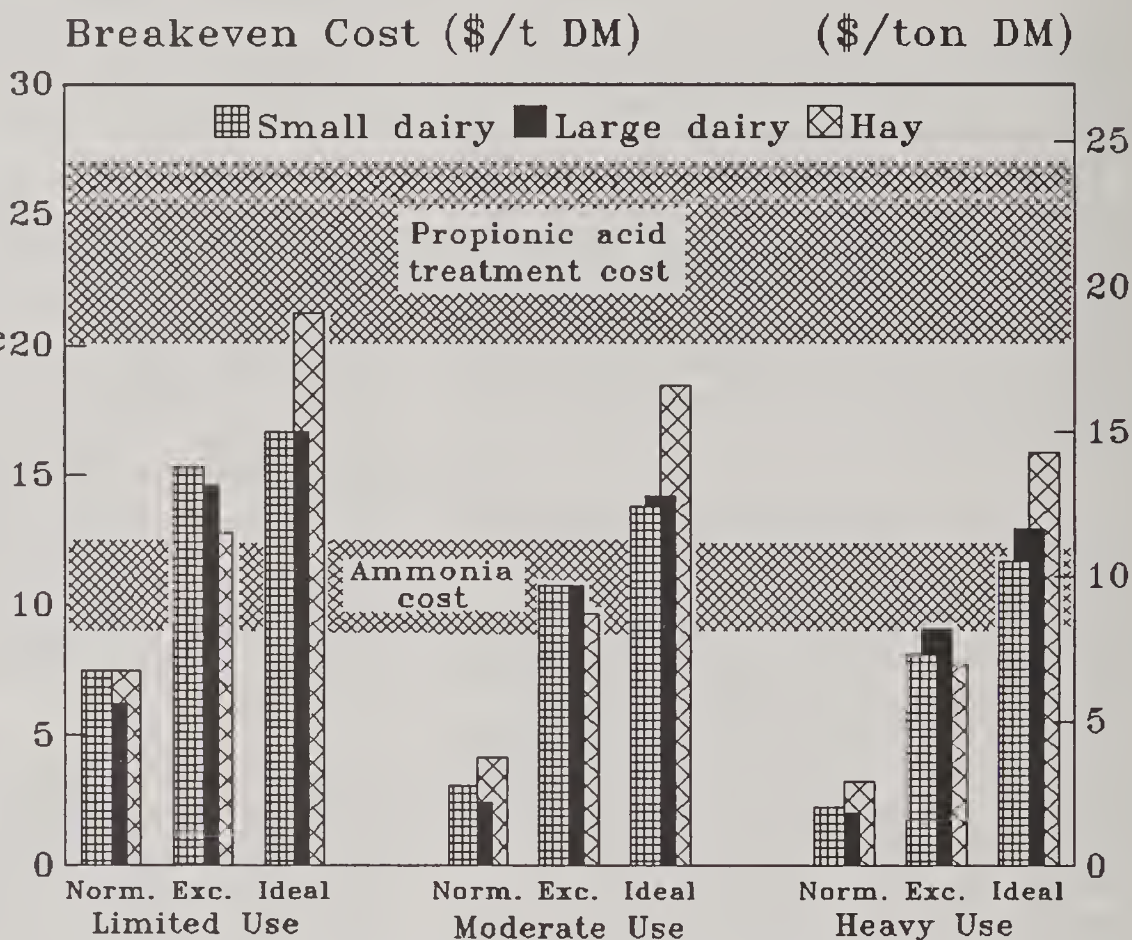


Figure 1. A comparison of the breakeven costs for different preservation treatments to current costs of propionic acid and ammonia treatments of high-moisture hay.

Conclusion

The effectiveness of hay preservatives must be improved considerably and/or the cost must be substantially reduced for economical treatment of high-moisture hay.

AMBIENT AIR DRYING OF BALED HAY

C.A. ROTZ and H.A. MUHTAR

Introduction

Hay growers are interested in baling hay before field curing is complete to reduce harvest losses. Hay baled between 20 and 25% moisture (wet basis) is often treated with a chemical or biological additive to reduce deterioration in storage. Chemical treatments such as propionic acid can reduce mold and heating in hay, but dry matter and nutrient losses during storage are high compared to dry hay. Biological treatments have not improved hay preservation.

Another approach to high-moisture hay harvest is to dry the hay after it is baled. Hay can be dried by forcing unheated

ambient air through a hay stack with a fan and plenum. After 4 to 6 weeks of fan operation, the hay is dry enough for stable storage. This technique is commonly used in Eastern Ontario, Canada. In order to properly evaluate and compare this hay harvest technique to other methods, information was needed on dry matter and nutrient losses during storage. An experiment was conducted to measure heating, dry matter loss and nutrient changes in hay stored on farms that routinely use the process.

Materials and Methods

Hay was baled, dried and stored on two cooperating dairy farms in Ontario using their normal procedures. First cutting alfalfa hay (10 to 20% timothy) was baled on June 4 and stacked in the hay barn. The driers used on the two farms were similar with a plenum under the center of the stack. Hay stacked beside the plenum was placed on pallets to allow air to flow at a uniform pressure under the stack. A 5 horsepower fan forced ambient air through the stack. Each unit was capable of drying up to 100 tons of hay. The fans were operated for the first 4 weeks of storage until the hay was dry enough for stable preservation.

Measurements were made on bales placed inside the hay mows. On farm 1, ten bales were monitored. A hay sample was collected from each bale, and the bales were labeled, weighed and placed in the mow. On farm 2, sixteen bales were monitored with eight of these bales treated with a bacterial hay preservative. Since the bacterial treatment was normally used on this farm, the monitored bales were stacked inside a mow of treated hay. The moisture contents of the monitored bales on farm 2 were higher than that normally used. Wetter hay was used to test the process under more extreme conditions. The moisture content of the hay surrounding the monitored bales was about 25%. Ambient air temperature and bale temperatures were measured every 6 h.

After 4 months, the monitored bales were removed from storage. They were weighed, sampled with the boring device and observed for mold. Hay samples were dried at 60°C for 72 h to measure dry matter content. Crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin and acid detergent insoluble protein (ADIP) were measured with standard laboratory procedures. Data collected on losses and nutrient changes had to be compared to those from hay stored by conventional methods. Since conventional methods were not available on the farms, the data were compared to those from other experiments with similar conditions.

Results and Discussion

Initial moisture content of individual bales ranged from 20 to 35%. All hay used in the trials was good quality dairy hay. Bale densities ranged from 95 to 170 kg DM/m^3, and densities were a little greater on farm 1. The hay was drier than expected when removed from storage (9 to 11% moisture) which implies that the forced air method can dry hay more than occurs with natural convection. Little or no heating occurred in most of the monitored bales. Bale temperature was within a couple degrees of the ambient air temperature throughout the experiment. Hay temperature varied a few degrees during the day, and the variation lagged a few hours behind the daily variation in air temperature.

Dry matter loss was similar to that expected in dry hay and considerably less than that in wet hay (Table 1). Losses measured in individual bales varied from -1 to 8% and averaged about 3%. Since fiber is not lost during storage, fiber concentration must increase in proportion to the loss of non-fiber constituents. Measured increases in fiber concentration were somewhat greater than those expected in dry hay, but less than those expected in conventionally stored wet hay (Table 1).

Crude protein is lost at a lower rate than other dry matter during storage. With the differing rates of loss, a very small gain in CP concentration is expected in dry hay with a little higher gain in conventionally stored wet hay (Table 1). The measured

change in CP concentration was very small, similar to that normally found in dry hay. A large increase in ADIP concentration can occur in wet hay when heating occurs. Since the hay remained near the ambient air temperature in this experiment, the concentration of ADIP did not change. Dry matter and nutrient losses were less than those reported for high-moisture hay treated with chemical or bacterial hay preservatives.

Conclusion

Although two hay storage trials are not conclusive evidence, they indicate that drying moist hay with forced ambient air can provide effective preservation, similar to that in field cured dry hay.

Table 1. Property changes during the storage of high-moisture hay dried with ambient air compared to typical changes that occur in dry hay and untreated high-moisture hay.

		Farm 1		Farm 2		
	Hay baled dry*	Dried	Moist	Dried	Moist	Treated*
Moisture content (%)	16.0	23.7	23.7	30.5	30.5	28.1
Weight loss (%)	7.4	17.1	19.4	25.0	28.9	24.4
Dry matter loss (%)	3.0	3.2	6.3	2.7	10.0	3.8
CP increase (% DM)	0.3	-0.3	0.7	0.2	1.2	0.4
ADF increase (% DM)	1.0	1.0	2.2	1.4	3.7	2.3
NDF increase (% DM)	1.4	2.6	3.0	2.1	4.9	3.3
ADIP increase (% DM)	0.0	0.6	9.4	-0.6	25.0	0.6

*Treated with a bacterial hay preservative and dried with ambient air.

ROTARY POWER REQUIREMENTS FOR AGRICULTURAL EQUIPMENT

C.A. ROTZ and H.A. MUHTAR

Introduction

Power requirement data are used by machinery managers to match tractors and implements for efficient and cost effective farming operations. Power data are also used to model machine performance and fuel consumption in farming system models such as DAFOSYM. The American Society of Agricultural Engineers (ASAE) publishes power data for use by machinery managers and modelers, but the data have not been revised for 15 years. Periodic updating is required due to changing farming practices and machinery designs. A study was undertaken to review current information on rotary power and to summarize relevant data in a table for convenient reference. Rotary power is defined as the functional power required at the implement engine or tractor PTO shaft to drive the implement.

Materials and Methods

The model developed to predict average rotary power requirements for all major farm machines is:

$$P_r = a + bW + cF$$

Where P_r is the rotary power required (kW or hp), W is the working width of the machine (m or ft), F is the material throughput (t/h or ton/h wet basis) and *a, b, and c* are machine specific parameters. The major effort in model development was determining parameters for each machine. Because available power information differed across machine types, a generic procedure for parameter estimation was not possible. The primary source for new power data was the Prairie Agricultural Machinery Institute. This institute has evaluated many agricultural machines and reported power requirements. Information from textbooks and published research was also used. In some cases, information was provided by equipment manufacturers. In all cases, parameter values were set based upon all information available. A value selected was not necessarily the mean of available data, but rather the selection of a value which seemed most reasonable for typical conditions. Because power requirements vary with machine design, machine adjustment, machine age and crop conditions, a range in parameter values was determined.

Results and Discussion

Typical, average rotary power parameters are summarized in Table 1 for all major types of agricultural machines. The typical values are listed along with an expected range or variation due to differences in machine design, machine condition and crop characteristics. Typical values can be adjusted within this range when conditions are likely to cause a substantial increase or decrease from the normal power requirement. Along with the power equation, this table provides a convenient reference for determining power requirements.

Conclusion

The new power model is being integrated into DAFOSYM to improve the simulation of machine performance and fuel use. The model and parameters are under review for revision of the power data published in the ASAE Standards.

Table 1. Parameters for determining rotary power requirements of agricultural equipment with expected range in average power requirement due to differences in machine design, machine adjustment and crop conditions.

Machine type	Parameter			Parameter			Range
	a kW	*b* kW/m	*c* kWh/t	*a* hp	*b* hp/ft	*c* hph/T	±%
Baler, small rectangular	2.0	0	1.0*	2.7	0	1.2*	35
Baler, large rectangular bales	4.0	0	1.3	5.4	0	1.6	35
Baler, large round (var. chamber)	4.0	0	1.1	5.4	0	1.3	50
Baler, large round (fix. chamber)	2.5	0	1.8	3.4	0	2.2	50
Beet harvester[‡]	0	4.2	0	0	1.7	0	50
Beet topper	0	7.3	0	0	3.0	0	30
Combine, small grains	20.0	0	3.6[§]	26.8	0	4.4[§]	50
Combine, corn	35.0	0	1.6[§]	46.9	0	2.0[§]	30
Cotton picker	0	9.3	0	0	3.8	0	20
Cotton stripper	0	1.9	0	0	0.8	0	20
Feed mixer	0	0	2.3	0	0	2.8	50
Forage blower	0	0	0.9	0	0	1.1	20
Flail harvester, direct-cut	10.0	0	1.1	13.4	0	1.3	40
Forage harvester, corn silage	6.0	0	3.3[ǀ]	8.0	0	4.0[ǀ]	40
Forage harvester, wilted alfalfa	6.0	0	4.0[ǀ]	8.0	0	4.9[ǀ]	40
Forage harvester, direct-cut	6.0	0	5.7[ǀ]	8.0	0	6.9[ǀ]	40
Forage wagon	0	0	0.3	0	0	0.3	40
Grinder mixer	0	0	4.0	0	0	4.9	50
Manure spreader	0	0	0.2	0	0	0.3	50
Mower, cutterbar	0	1.2	0	0	0.5	0	25
Mower, disk	0	5.0	0	0	2.0	0	30
Mower, flail	0	10.0	0	0	4.1	0	40
Mower-conditioner, cutterbar	0	4.5	0	0	1.8	0	30
Mower-conditioner, disk	0	8.0	0	0	3.3	0	30
Potato harvester[‡]	0	10.7	0	0	4.4	0	30
Potato windrower	0	5.1	0	0	2.1	0	30
Rake, side delivery	0	0.4	0	0	0.2	0	50
Rake, rotary	0	2.0	0	0	0.8	0	50
Tedder	0	1.5	0	0	0.6	0	50
Tub grinder, straw	5.0	0	8.4	6.7	0	10.2	50
Tub grinder, alfalfa hay	5.0	0	3.8	6.7	0	4.6	50
Windrower/swather, small grain	0	1.3	0	0	0.5	0	40

*Increase by 20% for straw.

[‡]Total power requirement must include a draft of 11.6 kN/m (_40%) for potato harvesters and 5.6 kN/m (±40%) for beet harvesters. A row spacing of 0.86 m for potatoes and 0.71 m for beets is assumed.

[§]Based upon material-other-than-grain (MOG) throughput for small grains and grain throughput for corn. For a PTO driven machine, reduce parameter *a* by 10 kW.

[ǀ]Throughput is units of dry matter per hour with a 9 mm (0.38 in.) length of cut. At a specific throughput, a 50% reduction in the length of cut setting or the use of a recutter screen increases power 25%.

REPAIR AND MAINTENANCE COST DATA FOR AGRICULTURAL EQUIPMENT

C.A. ROTZ and W. BOWERS

Introduction

Maintenance and repair costs are a relatively important part of the total cost of owning and operating agricultural equipment. Accurate prediction of the trend for increasing repair and maintenance costs is critical to determine the economic optimum life or best time to trade machines. Repair and maintenance costs are also important for making accurate cost/return budgets in models such as DAFOSYM. As a guide, machinery managers and modelers rely on repair and maintenance cost parameters published by the American Society of Agricultural Engineers (ASAE). As new machines are developed or machines are redesigned, repair and maintenance cost parameters change. The published parameters must be reviewed periodically to assure their relevance to current machines. A study was undertaken with the objective of using a thorough review by machinery experts to update the repair and maintenance data published in the ASAE Standards.

Materials and Methods

The accepted standard model of repair and maintenance costs published by ASAE is:

$$C_{rm} = RF1\ (P)\ (h)^{RF2}$$

where C_{rm} is the accumulated repair and maintenance cost ($), P is the machine price ($), h is the accumulated use of the machine (1000 hours) and RF1 and RF2 are machine specific repair and maintenance parameters. Machinery repair and maintenance information was reviewed to verify or revise the machine specific parameters. Due to the lack of published data, the review was heavily based upon the opinions of machinery experts from industry and public service. A comparison of predicted repair costs across machine types was used to further verify the parameters.

Results and Discusion

As a result of the review, estimated life was increased for tractors, planting equipment, and self-propelled harvesting equipment and decreased for beet harvesting equipment and forage blowers (Table 1). Major reductions in the repair and maintenance cost parameters were made for tractors, moldboard plows, planting equipment, self-propelled combines, mower-conditioners, rakes, forage harvesters, cotton harvesters and fertilizer spreaders. Parameters were added for rotary mowers and mower-conditioners, self-propelled windrowers, and large-bale balers. The final parameters listed in Table 1 provide a guide for general use by engineers, economists and others involved with machinery management. These parameters along with the equation predict repair and maintenance costs which are within 25% of actual costs for the average machine.

Conclusion

The revised parameters are under review by the Machinery Management Committee of ASAE for publication in the ASAE Standards. DAFOSYM has been revised to include the new parameters for more accurate simulation of machinery costs.

Table 1. Current and proposed new values for wear-out life and repair and maintenance cost parameters for agricultural equipment.

Machine	Wear-out life (h)		Total life repair cost(%)		Repair cost parameters*	
	Current Standard	New Value	Current Standard	New Value	RF1	RF2
TRACTORS						
2 wheel drive & stationary	10000	12000	100	100	0.007	2.0
4 wheel drive & crawler	10000	16000	100	80	0.003	2.0
TILLAGE & PLANTING						
Moldboard plow	2000	2000	150	100	0.29	1.8
Heavy-duty disk	2000	2000	60	60	0.18	1.7
Tandem disk harrow	2000	2000	60	60	0.18	1.7
Chisel plow	2000	2000	80	75	0.28	1.4
Field cultivator	2000	2000	80	70	0.27	1.4
Spring tooth harrow	2000	2000	80	70	0.27	1.4
Roller/mulcher-packer	2000	2000	40	40	0.16	1.3
Rotary hoe	2000	2000	60	60	0.23	1.4
Row crop cultivator	2000	2000	100	80	0.17	2.2
Row crop planter	1200	1500	80	75	0.32	2.1
Grain drill	1200	1500	80	75	0.32	2.1
HARVESTING						
Corn picker sheller	2000	2000	70	70	0.14	2.3
Combine	2000	2000	60	60	0.12	2.3
Combine, self-propelled	2000	3000	35	40	0.04	2.1
Mower	2000	2000	150	150	0.46	1.7
Mower, rotary		2000		175	0.44	2.0
Mower-conditioner	2000	2500	80	80	0.18	1.6
Mower-conditioner, rotary		2500		100	0.16	2.0
Windrower, self-propelled		3000		55	0.06	2.0
Side delivery rake	2000	2500	100	60	0.17	1.4
Rectangular baler	2000	2000	80	80	0.23	1.8
Large rectangular baler		3000		75	0.10	1.8
Large round baler		1500		90	0.43	1.8
Forage harvester	2000	2500	80	65	0.15	1.6
Forage harvester, self-prop.	2500	4000	40	50	0.03	2.0
Sugar beet harvester	2500	1500	70	100	0.59	1.3
Potato harvester	2500	2500	70	70	0.19	1.4
MISCELLANEOUS						
Fertilizer spreader	1200	1200	120	80	0.63	1.3
Boom-type sprayer	1500	1500	70	70	0.41	1.3
Beet topper/stalk chopper	2000	1200	60	35	0.27	1.4
Forage blower	2000	1500	50	45	0.22	1.8
Forage wagon		2000		50	0.16	1.6
Wagon	3000	3000	80	80	0.19	1.3

VALUE-ADDED PRODUCTS FROM FORAGE

R.G. KOEGEL, R.J. STRAUB and J.Y. KIM

Introduction

Wet fractionation of herbage consists of extensive cell disruption followed by juice expression. This results in a high moisture-low fiber fraction and a low moisture-high fiber fraction both of which may be better suited to particular uses than the original herbage. In some cases the high liquid fraction may be further fractionated to yield higher value products.

Workers at the U.W. Biotechnology Center have succeeded in inserting a gene into alfalfa which causes it to produce a substance not normally produced. They hypothesize that transgenic alfalfa could be used as a "factory" to produce industrially valuable substances, such as enzymes. These substances would be obtained from the transgenic alfalfa by wet fractionation followed by separation and purification of the liquid fraction.

In order for this approach to be competitive with more conventional fermentation vat production, highly efficient procedures for wet fractionation and subsequent purification are necessary.

Materials and Methods

Work has proceeded in four areas: (1) cell disruption techniques compatible with high throughput and low specific energy requirement, (2) quantifying and modeling the juice expression process, (3) juice processing including clarification, concentration and stabilization to make subsequent purification in a central facility technically and economically feasible, and (4) study of economic feasibility of this approach.

Result and Discussion

Extraction strategies: Results of the extraction work have shown impact to be an effective maceration method when compared to other techniques on the basis of juice recovery. Moisture content change of the impact macerated alfalfa under constant

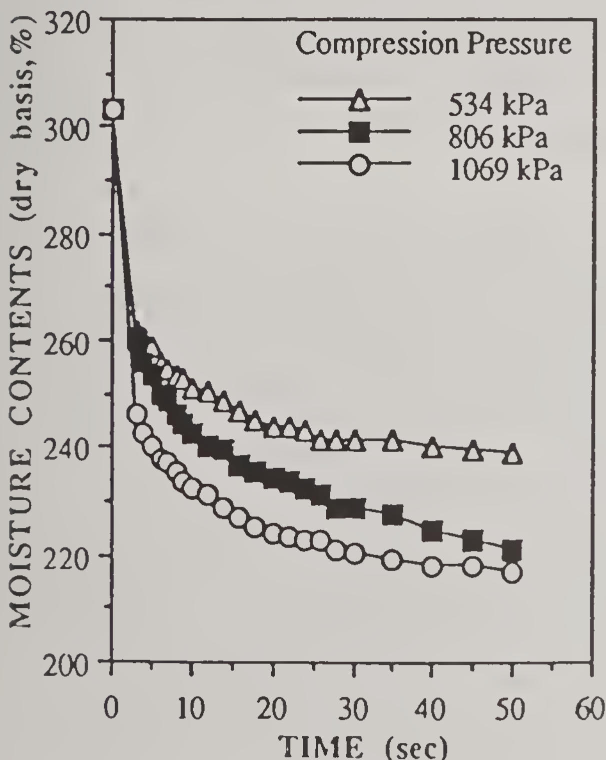


Figure 1. Moisture contents vs. time of impact macerated alfalfa.

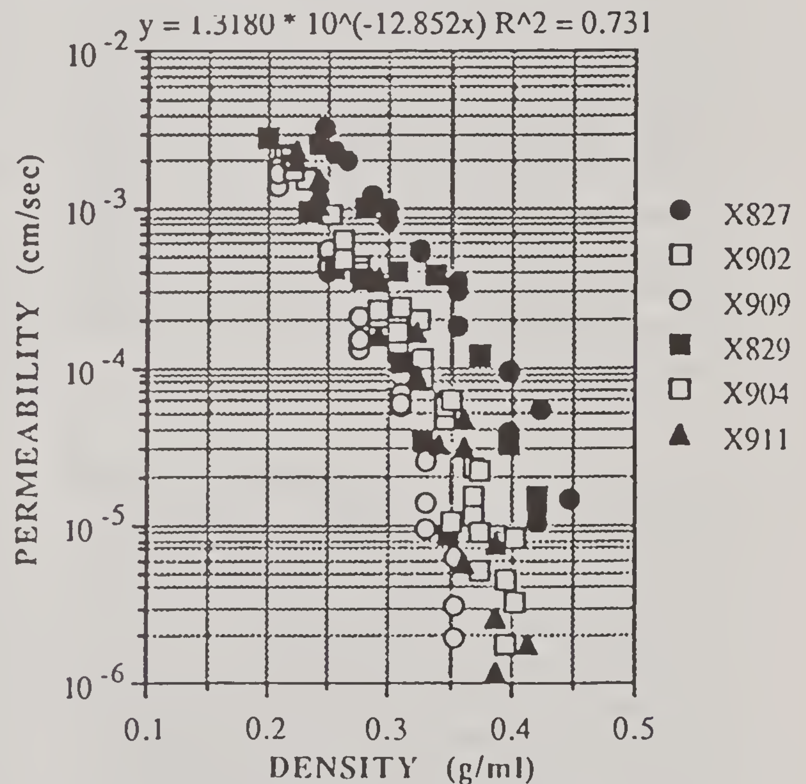


Figure 2. Permeability vs. dry bulk density of impact macerated alfalfa.

pressure conditions is shown in Figure 1. Note that, for the pressure conditions evaluated, most of the change in moisture (or weight) occurred in the initial three seconds of expression. Increasing pressure results in greater changeover time, but at a decreasing rate due to reduced permeability or blinding.

The quantity of juice which can be expressed from macerated plant material in a practical, large-scale operation is limited by physical properties of the plant material itself. The rate of juice expression approximates Darcy's Law for flow in porous media: Flow velocity is directly proportional to the pressure applied and to the permeability of the media. As pressure is applied, however, the plant material consolidates, drastically reducing the permeability. Figure 2 shows the relationship between bulk dry matter density of the plant material and permeability. As bulk density doubles, permeability is reduced by more than two orders of magnitude. This effectively reduces the juice flow rate to a level incompatible with operations involving high throughput. The minimum practical level to which moisture content can be reduced is thus around 200% (dry basis).

Model development for juice extraction: Modeling work being evaluated involves a simplified prediction model based on an experimentally determined "characteristic moisture" which would encompass a number of variables including material properties, pressing variable, and maceration method. Knowing the initial moisture and the characteristic moisture would allow for use of the model to predict dewatering performance.

Juice processing: Concentration and stabilization of juice at the point of origin is a technical and economic necessity for subsequent purification of the target enzyme. Three separation processes have been partially evaluated to date. These include an electrostatic process, an electrodynamic process, and a steady-flow centrifugation process. The first two have shown little promise so far, while the third appears to have potential for removing a significant part of the particulate protein from the juice. At present, improved centrifugation equipment is being readied to determine the extent to which particulate (chloroplastic) protein can be removed from the juice. Such clarification may make possible the concentration of the remaining, largely soluble, protein by membrane technology. This clarified juice concentrate would then have to be stabilized for transport by refrigeration or other means to a central processing facility where the final separation of the target enzyme would take place.

Economic feasibility studies: Further refinement has taken place on the economic feasibility analysis. This continues to show the concentration of the target enzyme in the juice as the most critical variable for economic viability. While the cost of separation is also critical, this is, to a large extent, also a function of the target enzyme concentration. Another requirement for economic viability is on-farm concentration and stabilization of the juice. This is necessary to avoid excessive transportation costs and the creation of an unmanageably large waste stream at a centralized processing facility. It is anticipated that the juice will be produced at approximately 25 tons/hour. Since proteolysis begins immediately upon juice expression, stabilization is also necessary to avoid excessive losses of the target enzyme as well as other valuable protein.

Conclusions

While it would be premature to speculate on overall feasibility of the production of industrially valuable substances in plants such as alfalfa, considerable progress has been made in a number of areas, and so far no insurmountable obstacles have been encountered. The key to economic feasibility of this technology appears to be the concentration of the target substance which can be produced in transgenic plants. In addition to the target substance, a relatively larger quantity of protein concentrate valuable in the rations of both ruminants and monogastrics will be produced. Thus, at least two "value-added" products will result from this endeavor.

TEMPERATURE EFFECTS ON PROTEOLYSIS IN ALFALFA SILAGE

R.E. MUCK

Introduction

Proteolysis, the breakdown of protein to soluble non-protein nitrogen fractions, during ensiling reduces the quality of the crude protein in alfalfa silage. This loss in nutritional value is particularly a problem in feeding high-producing, lactating dairy cows.

One factor affecting proteolysis is temperature. Within normal ensiling temperatures, proteolysis rate increases with temperature. However, higher temperatures also speed fermentation, lowering pH which in turn reduces proteolytic activity. From an earlier study, it would appear that the amount of proteolysis that occurs during ensiling increases with temperature for temperatures below 25°C but is relatively unaffected by higher temperatures. In that study, only final silage quality was measured. In the current study, effects of temperature on the rates of proteolysis during fermentation were measured.

Methods

Alfalfa was harvested with normal field equipment in second (July) and fourth (October) harvests and ensiled at two dry matter levels (35 and 55% DM) in mini-silos (100 ml centrifuge tubes). The silos at each DM level were divided among four water baths set at 10, 20, 30 and 40°C, respectively. Two silos of each treatment were opened and analyzed at 0.5, 1, 2, 4, 8, 16 and 32 d of ensiling. The initial alfalfa and silages were analyzed for total Kjeldahl nitrogen, trichloroacetic acid-soluble non-protein nitrogen (NPN), ammonia, free amino acids, pH, moisture and fermentation products.

Results and Discussion

Similar to the earlier study, the total amount of proteolysis during ensiling, as indicated by soluble NPN (Table 1), increased with temperature at the lower temperatures (10, 20 and 30°C) but was unaffected by temperature at higher temperatures (30 *vs.* 40°C). This plateauing of proteolysis at higher temperatures did not appear to be from the breakdown of all protein available for proteolysis as implied by the 55% DM results in second harvest. The plateau is more likely from temperature effects on the decline in proteolytic activity.

Proteolytic activity during fermentation was estimated by fitting the soluble NPN *vs.* time data for a given treatment and trial to an appropriate equation, usually a two- or three-term exponential equation. The first derivatives of these equations provided proteolysis rates at any time during fermentation. The proteolysis rate at the start of ensiling within a cutting and DM level was exponentially correlated with the inverse of the absolute temperature (Fig. 1). Also the initial proteolysis rate was consistently

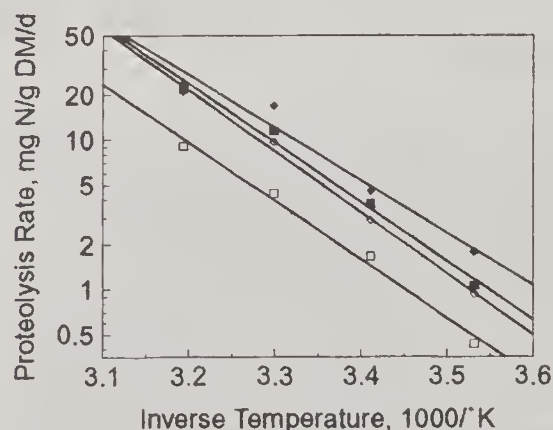


Figure 1. Initial proteolysis rates as a function of temperature. Closed symbols - 35% DM; open symbols - 55% DM; ■ - July; ♦ - October.

lower in the high DM silages. These findings are in agreement with other studies.

Adjusting the proteolysis rates for pH and normalizing rates with respect to the initial rate, proteolysis rates declined exponentially the first 2 to 8 days (Fig. 2). Thereafter rates dropped more slowly. Normalized rates dropped most rapidly at 40°C and least at 10°C, implying a more rapid loss of enzyme activity at higher temperatures. After 32 days ensiling, the proteolysis rate at 10°C was more than 10% of the initial rate whereas proteolysis rate was less than 1% of the initial rate at 40°C. The normalized rates were unaffected by DM content.

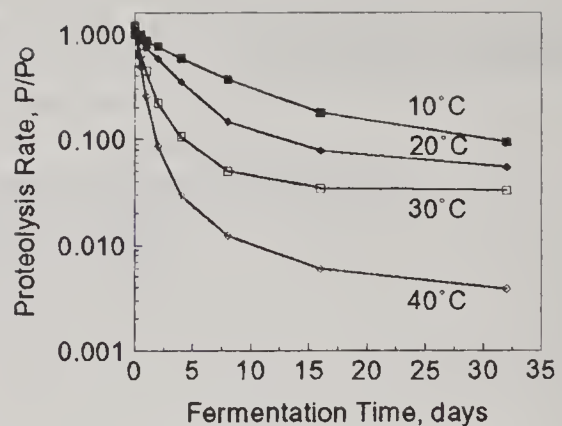


Figure 2. Average proteolysis rates during ensiling adjusted to pH 6 and normalized relative to initial proteolysis rate.

Conclusions

These results suggest that typical silage temperatures in Wisconsin during the summer probably have little effect on the amount of proteolysis that is observed. However, in October when air temperatures are cooler, maintaining the silage as cool as possible should result in reduced levels of protein breakdown in alfalfa silage. Finally, the slower decline in proteolysis rates at low temperatures suggests that attempts to reduce proteolysis through improved fermentation rate and/or quality of fermentation are more likely to be successful at low temperatures than at high ones.

Table 1. Soluble NPN (mg crude protein/g DM) in alfalfa ensiled 32 d as affected by silo temperature.

DM Level	Harvest	10#C	20#C	30#C	40#C
36	2	67	95	134	128
30	4	110	112	124	127
55	2	41	59	82	86
54	4	94	122	138	136

STREPTOCOCCUS BOVIS AS A SILAGE INOCULANT

B.A. JONES, R.E. MUCK and S.C. RICKE

Introduction

Maximizing forage nutrients during ensiling depends on a rapid and sufficient pH decline. Bacterial inoculants should help attain a rapid pH decline but have been inconsistent for two reasons. First, inoculants have been ineffective when bacterial substrates in the plant are low. Secondly, inoculants will be effective only when they are capable of overcoming the epiphytic bacterial population of the alfalfa. *Streptococcus bovis*, a homolactic bacterium, was tested as a bacterial inoculant because of its ability to utilize starch, a plant substrate unavailable to other lactic acid bacteria, and because its doubling time is 30% shorter than most lactic acid bacteria thus enabling it to better overcome the epiphytic flora.

Materials and Methods

Four strains of *Streptococcus bovis* were evaluated for growth kinetics, starch utilization and ability to lower the pH quickly of

alfalfa silage. One strain was selected and compared to *Enterococcus faecium*, a fast growing lactic acid producing bacterium commonly used in bacterial inoculants. Testing was completed over three studies (1 in 1988, 2 in 1989) using wilted alfalfa (28-34% DM). Treatments were control (C), *Enterococcus faecium* (F), *E. faecium* and commercial inoculant (F + CI), *S. bovis* (B), *S. bovis* and commercial inoculant (F + CI), and commercial inoculant (CI). Replicate laboratory silos were emptied and analyzed at 0.5,1,2,4,8 and 40 days for pH, fermentation products and nitrogen fractions.

Results and Discussion

The decline of silage pH over time was different for the three studies (Figure 1). In general, the treatments containing the commercial inoculant obtained the most rapid pH decline, but without commercial inoculant B consistently dropped pH faster than C or F. Due to the difference in pH decline of the 1988 study, this was analyzed separately from the 1989 studies.

Streptococcus bovis was beneficial in lowering pH and acetate content early in the fermentation in the 1988 study but no effect was observed after 1 day. There was no consistent trend from adding *S. bovis* to the commercial inoculant, although acetate was lower in the S. bovis plus commercial inoculant than the other commercial inoculant treatments at 40 d ($0.10 < p < 0.17$). Lack of effectiveness of *S. bovis* in this study may be related to the high numbers of epiphytic lactic acid bacteria on the alfalfa compared to the inoculants (10^7 and 10^5 bacterial/ g herbage, respectively).

Streptococcus bovis was more effective in the two studies completed in 1989. Most silage characteristics were significantly improved by *S. bovis* (B) when compared to C and F throughout the fermentation; however, the most dramatic effect was early (Table 1). Likewise, *S. bovis* did improve the silage characteristics when compared to the commercial inoculant alone (lower pH, acetic acid and NH_3-N) during the early time points. The addition of *S. bovis* to the commercial inoculant (B + CI) was beneficial relative to the other treatments receiving commercial inoculant (F + CI and CI) in lowering the pH and improving the organic acid content at the onset of the fermentation. The benefits noted in the 1989 studies demonstrate that *S. bovis* can be useful as an silage inoculant on wilted alfalfa.

Utilization of alfalfa starch by *S. bovis* was not demonstrated in any of the studies even though the selected strain was capable of utilizing soluble starch. Reasons for the discrepancy are unknown.

Conslusion

Streptococcus bovis, due to its faster growth rate and being homofermentative, was beneficial in initiating and improving the fermentation of wilted alfalfa. This effect was noted when *S. bovis* was used alone or combined with a commercial inoculant. Unfortunately, *S. bovis* did not use alfalfa starch as a substrate during ensiling. Even so, *S. bovis* still shows promise as an initiating organism in a silage inoculant.

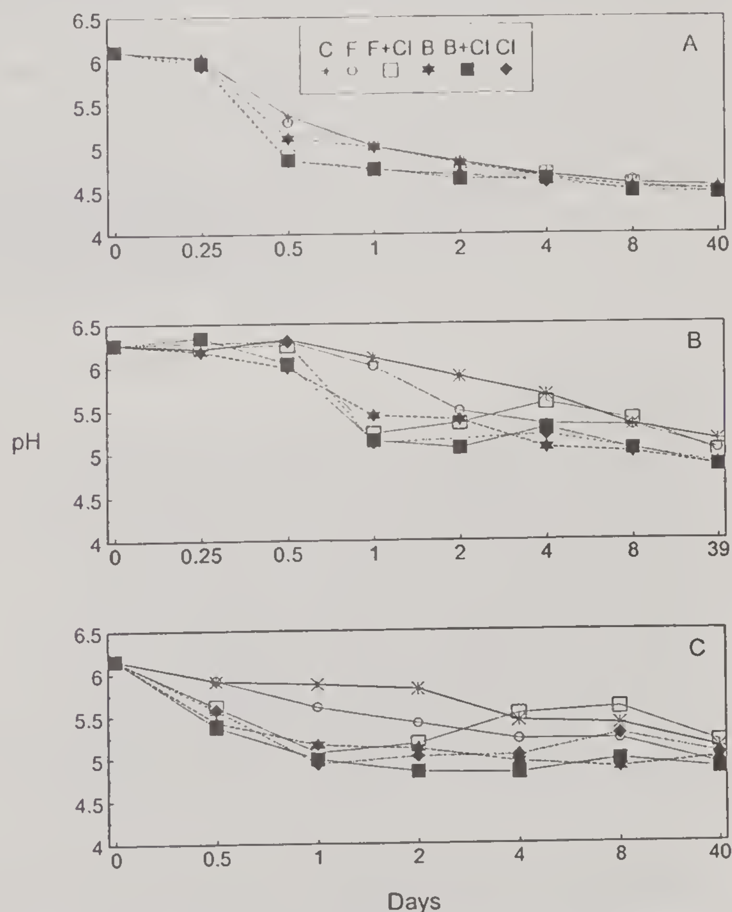


Figure 1. Effect of the inocula on pH decline of alfalfa silages from three studies. (A) 1988 study. (B) 1989-1 study. (C) 1989-2 study. Standard errors were 0.05-0.06 pH unit.

Table 1. Fermentation characteristics of treated alfalfa from the 1989 studies.[a]

Measurement[b]	Day	Control		*E. faecium*		*S. bovis*		*P*-value for the contrast				
		C	CI	F	F+CI	B	B+CI	B vs C	B vs F	B vs CI	B+CI vs CI	B+CI vs F+CI
pH	0.5	6.11	5.91	6.11	5.93	5.71	5.70	.01	.01	.01	.01	.01
NH_3-N		1.10	0.78	1.07	0.90	0.67	0.74	.05	.06	.45	.76	.56
Lactate		0.74	1.66	1.46	1.98	2.14	2.72	.01	.01	.03	.01	.01
Acetate		0.87	0.80	0.86	0.62	0.33	0.58	.01	.01	.01	.12	.68
pH	1	6.11	5.06	5.80	5.15	5.30	5.08	.01	.01	.02	.68	.45
NH_3-N		1.39	1.43	1.49	1.48	0.85	1.29	.03	.01	.03	.60	.48
Lactate		1.46	4.69	3.20	4.93	3.62	4.69	.01	.15	.01	.89	.55
Acetate		1.36	1.00	1.27	1.21	0.59	0.71	.01	.01	.04	.20	.01
pH	4.0	5.57	5.12	5.27	5.54	5.00	5.02	.01	.01	.13	.44	.01
NH_3-N		2.69	2.44	2.19	3.68	1.55	2.47	.01	.08	.02	.78	.01
Lactate		3.35	5.24	5.51	4.85	5.02	5.32	.01	.04	.47	.81	.26
Acetate		1.90	1.97	2.00	3.21	0.91	1.69	.01	.01	.01	.18	.01
pH	40	5.12	4.98	4.97	5.09	4.91	4.84	.03	.50	.67	.37	.03
NH_3-N		3.04	5.49	3.08	5.76	4.59	4.03	.11	.12	.54	.33	.13
Lactate		6.72	7.08	7.30	7.01	6.34	6.92	.55	.14	.25	.90	.94
Acetate		4.10	4.05	3.35	4.79	4.26	3.36	.80	.17	.92	.33	.04

[a]Values are the mean of the two studies completed in 1989.
[b]NH_3-N, ammonia nitrogen expressed as mg N/g of DM; lactate and acetate expressed as %DM.

EFFECT OF FERMENTATION AND INOCULATION ON ALFALFA CELL WALLS

B.A. JONES, R.D. HATFIELD and R.E. MUCK

Introduction

Ensiling of alfalfa is a major conservation method for the production of ruminant feeds in the United States. Research has suggested that the composition of the cell wall will change during ensiling ultimately altering the intake and/or digestibility of the forage by ruminants. This study was under taken to further evaluate the influence of fermentation and inoculation on cell walls of alfalfa.

Materials and Methods

Alfalfa, at three dry matter contents (19, 29 and 40% DM), was chopped manually, inoculated with lactic acid bacteria and ensiled into laboratory silos which were incubated at 30°C. Two levels of inoculation (< 10^4 and 10^6 bacteria/g herbage) were compared at each DM with the exception of the 19% DM alfalfa (< 10^4 only). Duplicate silos were opened and analyzed at 1, 2, 8 and 56 or 64 days.

Results and Discussion

Fermentation of the silages was typical for the 29 and 40% DM silages, but the 19% DM had only a decline in pH of 0.25 units. The high pH was associated with high acetate (5.3% DM) and ammonia (22% of

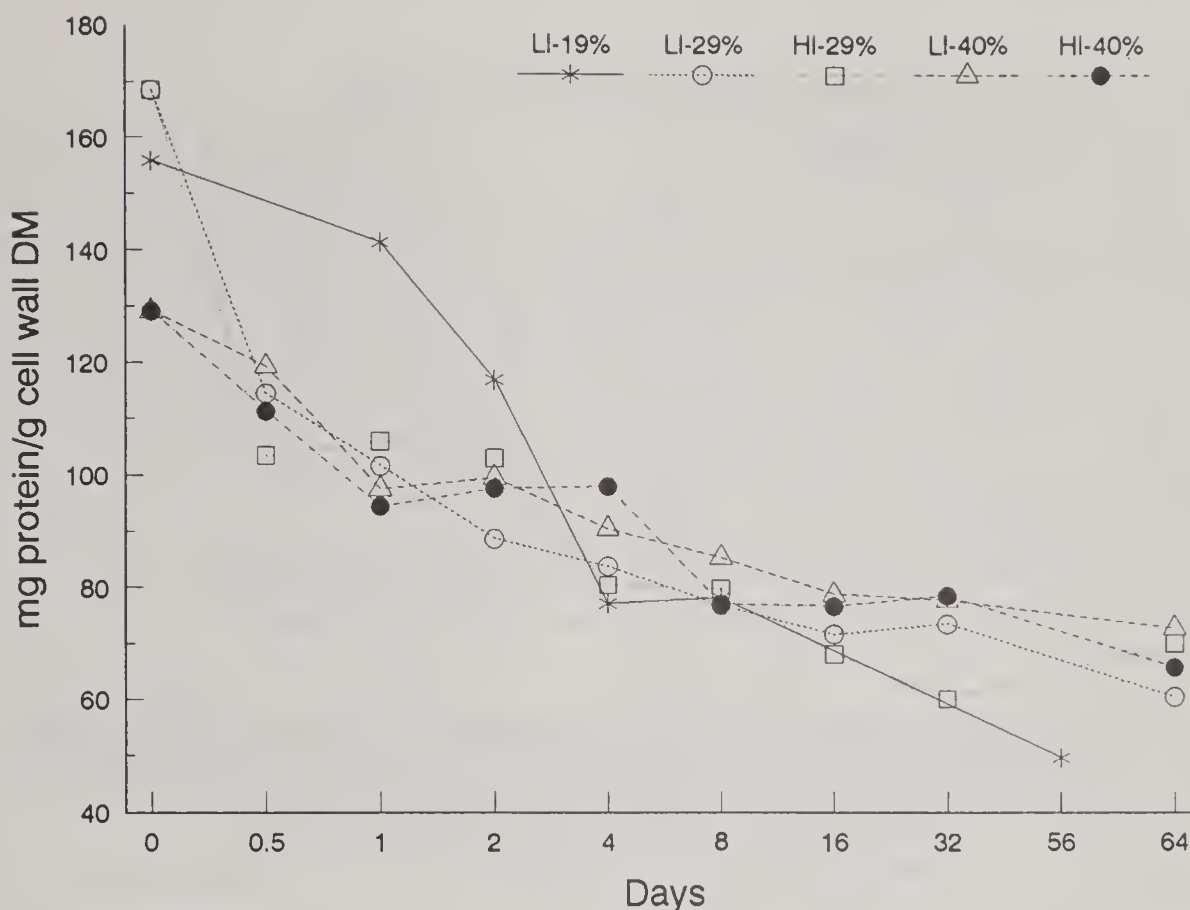


Figure 1. Decline in protein associated with the cell wall fraction of alfalfa ensiled at different inoculation rates and DM content. Legend: LI, low rate of inoculation; HI, high rate of inoculation; and 19, 29 and 40%, different DM contents.

total N) but not butyrate (< 0 .5% DM). Inoculation enhanced the pH decline early in the fermentation, but the pH values were not significantly different at the fermentation end point. Ensiling significantly reduced (46-68%, $p < 0.05$) protein associated with the cell wall in all silages regardless of inoculation rate (Figure 1). The lowest dry matter silage had the greatest protein loss. Protein contamination of lignin residues was also reduced during ensiling, but to a lower extent (< 40%).

The effect of ensiling on individual sugars in protein corrected cell walls varied. Cell wall uronics decreased by 12% during ensiling at 29% DM but were unchanged in the 19 and 40% DM. The arabinose and galactose contents of the cell wall were decreased in both 29 and 40% DM silages; rhamnose declined in the 29% DM silages only (Table 1). This reduction in arabinose and galactose was enhanced early in the fermentation with the high level of inoculation ($p < 0.05$); however like pH, there was no difference in the sugar concentration at the fermentation end point ($p > 0.32$). Xylose and glucose consequently increased due to the loss of the previously stated sugars. There was no change in the cell wall sugars of the 19% DM silage. Loss of sugars from the cell wall appears to be related to pH decline which results in acid hydrolysis of cell wall sugars.

Conclusion

Cell wall material is altered during ensiling. Protein associated with cell wall carbohydrates and lignin was significantly reduced during ensiling. This reduction of protein must be accounted for when evaluating cell walls or fiber components between alfalfa hays and silages or ensiling treatments. The cell wall sugars of arabinose, galactose, rhamnose and uronics decreased during ensiling with the extent varying with DM, sugar type and pH decline. Inoculation reduced the cell wall sugars only when the pH was lowered by inoculation. Reduction of the cell wall sugars appears to be principally by acid hydrolysis because losses of cell wall sugars were correlated with low pH values.

Table 1. Cell wall constituents of alfalfa herbage and silage.

	Days	Protein Contamination mg/g		mg/g cell walls[2]								
%DM	Ensiled	CWM[1]	Lignin	Lignin	Uronics	Fuc	Rha	Ara	Ga	Glu	Xyl	Man
19	0	157**	193**	158	120	4.9	19.0	44.6	25.8	364	83	19.3
	56	50	119	155	123	5.1	19.6	47.2	36.1	365	88	22.3
29	0	169**	145**	170	120*	2.9	13.6*	40.2**	27.5*	357*	104	23.5
	64	65	94	168	114	2.6	11.3	30.5	21.7	365	112	22.5
40	0	129**	147**	163	117	2.8	11.6	37.6*	26.0*	360	111	23.6
	64	70	134	167	119	2.6	11.9	31.6	22.4	374	111	22.1

[1]CWM = cell wall material.

[2]With protein contamination removed.

**, $P < .01$; *, $P < .05$ between initial forage and final silage within each DM content.

VALUE OF AMMONIUM PROPIONATE AND AMMONIUM FORMATE FOR PRESERVING ALFALFA SILAGE FED TO LACTATING DAIRY COWS

G.A. BRODERICK, D.B. RICKER and M.A. ELLIOTT

Introduction

Ease of mechanization and decreased field losses have made silage-making the method of choice for harvesting alfalfa fed to dairy cows in the U. S. However, when alfalfa is ensiled, typically 50 to 60% of the crude protein (CP) is present as nonprotein N (NPN). Silage NPN is used with lower efficiency by lactating cows; thus, improved protein efficiency would occur with reduced NPN formation in the silo. Nagel and Broderick (J. Dairy Sci. 75: 140-154, 1992) found that treating wilted alfalfa silage with formic acid reduced silage NPN by a third and increased production of milk by 3.4 kg/day and milk protein by 110 g/day when fed to cows in early lactation. Concern about cost, corrosion of machinery, and safety to those harvesting the silage, prevents formic acid from being used widely to reduce silage NPN. However, these results show that decreased NPN in alfalfa silage substantially increased its protein value. The objective of this research was to determine if treating wilted alfalfa silage with additives containing ammoniated propionic acid and formic acid (relatively safe forms of these acids) would reduce silage NPN and increase production of milk and milk components by lactating dairy cows.

Materials and Methods

Third-crop alfalfa was cut and wilted to approximately 35% DM and alternate loads ensiled into three upright concrete stave silos. Control (C) alfalfa silage was ensiled untreated. Alfalfa silage was treated at the silo blower with commercial additive containing ammoniated propionic acid (P; Cargill Storage Mate II) at a rate of 4 lbs/ton of wet material. Alfalfa silage also was treated at the silo blower with an experimental additive containing a mixture of ammoniated formic and propionic acids (FP), at a rate of 6 lbs/ton of wet material. Application rates for treated silages were equivalent to 3 lbs total acid/ton wet material. During ensiling, thermocouples were buried in each silo. Twenty-four cows in the second or later lactation, averaging 66 days-in-milk and 39.0 kg milk/day, were randomly assigned to diets in a 3X3 Latin square (a switchback design where each cow eventually gets each diet over the course of the experiment) and fed diets containing one of

the three silages. In addition, six rumen cannulated cows in mid-lactation were assigned to 3X3 Latin squares and fed the same three diets. Each group of cows was fed its respective diet for 3-week periods before switching to the next diet (total nine weeks). Milk production data are from the last two weeks of each period. Each diet contained (DM basis) 73% alfalfa silage, 26% high moisture corn, plus supplemental vitamins and minerals (Table 1). No protein supplement was fed. Weekly composite samples of silages and high moisture corn were analyzed for CP, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent insoluble N (ADIN), pH, ammonia and NPN. Recovery of DM was estimated from ash concentrations in composite silage samples and fresh forages.

Results and Discussion

Silage P (that treated with ammoniated propionic acid) had lower silo temperatures than the other two silages; pH was significantly lower in both silages P and FP (that treated with a mixture of ammoniated propionic and formic acids) than in C (control). These data indicated better fermentation with treatments P and FP and somewhat better preservation with P. Ash content and DM recovery also were numerically better than C with silages P and FP; these are not statistically significant. Concentrations of DM, CP, ADF, NDF and ADIN were not different among the three silages. Total NPN content of C was only 45% of CP, substantially lower than what we normally find with low DM alfalfa silage. Although NPN was not different, ammonia was greater in silage FP. Product FP contained about 13% ammonia, corresponding at the application rate used in this trial to 2.6% of CP. Ammonia level found in the silage FP was greater than C by .6% of CP. We concluded that lower temperature in P, and lower pH and greater DM recovery in both P and FP, indicated better fermentations were achieved in the treated silages. However, these treatments did not reduce NPN formation in alfalfa silage in this study.

Feed DM intake (DMI), body weight (BW) change and milk production data are in Table 2. Feeding of diets with treated alfalfa silages resulted in DMI which was 2.4 lbs/day greater than C. When DMI was expressed on the basis of % of body weight, intake was .11 (P) and .18 (FP) percentage units greater than C. Production of milk, fat, protein, lactose, and SNF were all greater than C with feeding of the treated silages; mean difference in these production traits was 5% higher than C. There were no differences between treated silages. Environmental temperatures during this trial (May 2 to July 4, 1991) were substantially above normal. Preservative treatment of silages may have prevented some of the depression in DMI which occurred with silage C, and thus supported greater milk yields. Changes in body weight and feed efficiency (milk/DMI) were not significantly affected by silage. Rumen ammonia was consistently higher with feeding of silages P and FP than C; this may partly reflect the ammonia added to treated silages.

Summary and Conclusion

The increasing use of silage-making for harvesting alfalfa has stimulated interest in improving utilization of silage nutrients. Lower mean silo temperature and pH, and greater DM recovery, indicated better fermentation and preservation of alfalfa silage treated with ammoniated propionic acid or a mixture of ammoniated formic and propionic acids. The ammonia added with the mixture of ammoniated acids led to a small but detectable increase in silage ammonia. There was no difference in NPN content of alfalfa silages; therefore, neither treatment appeared to reduce breakdown of alfalfa protein in the silo. Production of milk and all milk components was clearly greater than control with feeding of alfalfa silage treated with ammoniated propionic acid or the mixture of ammoniated formic and propionic acids. Increased production was due partly to increased feed intake at the high temperatures during the trial.

Table 1. Composition of Experimental Diets.

Ingredient	Diet C (Control)	Diet P (PA-Treated)	Diet FP (FA&PA-Treated)
		(% of DM)	
Alfalfa Silage	72.7	73.6	73.1
High Moisture Corn	26.0	25.1	25.6
DiCal	.7	.7	.7
TMS + Se	.5	.5	.5
Vitamins ADE Conc.	.1	.1	.1
Composition			
Crude Protein (%)	18.5	18.8	18.9
NE_l (Mcal/kg)	1.57	1.56	1.57
ADF (%)	25	25	25

TMS = trace-mineral salt; PA = ammoniated propionic acid; FA = ammoniated formic acid.

Table 2. Dry matter intake, BW change, and production of milk and milk components.

Item	Silage				STATS		
	Control (C) (A-4)	Propionic (P) (A-1)	Formic-Propionic (FP) (A-3)	SEM	Prob. TRT effect	Contrasts C vs. P & FP	P vs. FP
DM intake, kg/day	19.2	20.3	20.3	.2	.004	.001	.632
DM intake, % BW	3.24	3.35	3.42	.03	.009	.004	.177
BW change, kg/day	-.17	-.27	-.32	.13	.632	.476	.499
Milk, kg/day	29.4	30.7	30.9	.4	.096	.033	.961
3.5% FCM, kg/day	29.0	30.3	30.7	.4	.030	.010	.475
Fat, %	3.42	3.44	3.48	.04	.203	.167	.217
Fat, kg/day	1.01	1.05	1.07	.02	.030	.013	.261
Protein, %	2.75	2.73	2.77	.02	.362	.945	.158
Protein, kg/day	.81	.84	.85	.01	.166	.073	.480
Lactose, %	4.80	4.79	4.81	.02	.857	.591	.878
Lactose, kg/day	1.41	1.47	1.49	.02	.124	.044	.995
SNF, %	8.30	8.26	8.31	.03	.682	.792	.420
SNF/ kg/day	2.44	2.53	2.57	.03	.133	.047	.796
Milk/DM intake	1.54	1.55	1.51	.02	.239	.556	.107

BW = body weight: SNF = solids-not fat.

ARGININE AND LYSINE DEAMINATION BY A MONENSIN-SENSITIVE RUMINAL BACTERIUM, *CLOSTRIDIUM PEPTIDIVORANS*

J.A.S. VAN KESSEL and J.B. RUSSELL

Introduction

In the early 1960's, Bladen et al. showed that a variety of ruminal bacteria could deaminate amino acids, but the specific activities could not explain ammonia production *in vivo.* Strain SR, a recently isolated, monensin-sensitive, ruminal bacterium, grew rapidly on short peptides or amino acids. Although SR was present at low concentrations in ruminal fluid (10^7/ml), it had a very high specific activity of ammonia production (> 310 nmol of ammonia/mg of protein/min). Based on its capacity to produce ammonia, it appeared that SR could be a significant contributor to ruminal amino acid degradation, and its sensitivity to monensin provided an explanation for the "protein sparing" effects of ionophores. Strain SR deaminated arginine, lysine, serine and glutamine, but it grew most rapidly on arginine and lysine.

Streptococci, halobacteria, clostridia, eubacteria and mycoplasma ferment arginine by the arginine deiminase (ADI) pathway. Because this pathway of arginine metabolism yields only 1 ATP by substrate level phosphorylation, the energetics of arginine fermentation were curious. Recent work by Driessen et al., however, showed that *Lactococcus (Streptococcus) lactis* had an arginine/ornithine exchanger. Since neither a proton-motive force nor ATP was required for transport, it appeared that all of the ATP from substrate level phosphorylation could be used for growth. In *L. lactis,* lysine is taken up by the arginine/ornithine exchanger and by a separate system which is proton-motive force-driven.

Materials and Methods

Strain SR was grown in a medium which contained salts, vitamins, microminerals, Casaminoacids as a carbon source, and either lysine or arginine as an energy source. Deamination was monitored by the increase in ammonia. Transport of ^{14}C lysine and ^{14}C lysine was studied in whole cells and membrane vesicles which were artificially energized (potassium diffusion potentials and a chemical gradient of sodium). De-energized cells and membrane vesicles were also loaded with ornithine and diluted into buffers lacking ornithine to create an outwardly directed ornithine gradient.

Results and Discussion

Strain SR grew rapidly on arginine and lysine, but only if sodium was present. Arginine transport could be driven by either an electrical potential or a chemical gradient of sodium. Arginine was converted to ornithine, and it appeared that ornithine efflux created a sodium gradient which in turn drove arginine transport. There was a linear decline in arginine transport as pH was decreased from 7.5 to 5.5, and the cells did not grow at pH less than 6.0. The Eadie-Hofstee plot was biphasic, and arginine could also be taken up by a high capacity diffusion mechanism. Because arginine was a strong inhibitor of lysine transport and lysine was a weak inhibitor of arginine transport, it appeared that both lysine and arginine were taken up by an arginine/lysine carrier which had a preference for arginine. The rate of lysine fermentation was always proportional to the extracellular lysine

concentration, and facilitated diffusion was the dominant mechanism of lysine transport. When SR was grown in continuous culture on arginine or lysine, the theoretical maximum growth yield was similar (13 g cells/mol ATP), but the apparent maintenance energy requirement for arginine was greater than lysine (9.4 versus 4.4 mmol ATP/g cells/h). Based on differences in yield and maintenance energy, it appeared that active arginine transport accounted for approximately 40% of the total ATP.

The effect of pH on ruminal amino acid degradation has not been examined in a systematic fashion, but Erfle et al. noted that the ammonia production of mixed continuous cultures decreased when the pH was lowered. Previous work indicated that strain SR was only able to grow at near neutral pH, and this pH sensitivity is consistent with the effect of pH on the arginine carrier. Because lysine is often the first limiting amino acid, there has been considerable interest in the ruminal protection of this amino acid. SR was the only monensin sensitive ruminal bacterium which could ferment lysine at a rapid rate, but SR had a poor affinity for lysine. Because the K_s was high, one might conclude that monensin would only protect lysine at high lysine concentrations. However, it should be realized that K_s/V_{max} is a better index of the utilization rate than K_s alone and that SR has a very high V_{max} for lysine. Based on these results it is possible that monensin could protect lysine as well as other amino acids from ruminal degradation.

THE EFFECT OF pH ON THE RESISTANCE OF RUMEN BACTERIAL MEMBRANES TO PROTON CONDUCTANCE

J.B. RUSSELL

Introduction

The rumen is well buffered by salivary secretions, but ruminal pH can decline significantly when animals are fed high-energy diets. When ruminal pH declines, there is a marked shift in the types of microorganisms which inhabit the rumen and fementation end-products. Rumen acidosis can decrease food intake and cause acute indigestion, but there was little information regarding the effect of pH on ruminal bacteria. The question arose, “why some some ruminal bacteria are resistant to low pH while others are sensitive.”

When extracellular pH declines, a bacterium is faced with two alternatives: 1) let intracellular pH decline, or 2) pump protons outwardly across the cell membrane to maintain a more or less constant intracellular pH. When protons are pumped from the cell, the inside of the cell is left negative relative to the outside. The inside of the cell becomes the cathode, and the outside acts as the anode. Although the cell membrane serves as a barrier to passive current flow, it is not a perfect insulator (resistor).

When protons re-enter the cell, heat is dissipated according to the simple equation, watts = volts x amperage. The rate of proton re-entry should be governed by Ohm’s law, wattage = voltage^2/resistance and amperage = voltage/resistance, but there have been few direct determinations of membrane resistance. In fermentative bacteria (e.g. streptococci), membrane current and voltage are dependent on the activity of membrane ATPases, and these ATPases are driven by the free-energy change of the fermentation. When ATP is hydrolyzed by the membrane bound ATPases, there is less energy to drive microbial growth (protein synthesis).

Materials and Methods

Non-growing cultures of *Streptococcus bovis* JB1 were incubated in 2-[N-moropholino] ethanesulfonic acid (MES)-

phosphate buffer (pH 6.8) and glucose (2 g/liter), and heat production was measured with a sensitive continuous flow calorimeter. Membrane voltage was estimated from the uptake of ^{14}C-labeled benzoic acid and tetraphenylphosphonium ion.

Results and Discussion

Non-growing cultures produced heat at a rate of 0.17 mW/mg protein, and this rate was proportional to the enthalpy change of the homolactic fermentation. Since the growth-independent heat production could be eliminated by dicyclohexylcarbodiimide (DCCD), an inhibitor of F_1F_0 ATPases, it appeared that virtually all of the energy was being used to counteract proton flux through the cell membrane. When the pH was decreased from 6.8 to 5.8, heat production and glucose consumption increased, the electrical potential ($\Delta\Psi$) declined, the chemical gradient of protons (ZΔpH) increased, and there was a small increase in total protonmotive force (Δp). Further decreases in pH (5.8 to 4.5) caused a marked decrease in heat production and glucose consumption even though there was only a small decline in membrane voltage.

Based on the enthalpy of ATP (4 kcal or 16.8 kj/mol), it appeared that 38% of the wattage was passing through the cell membrane. The relationship between membrane voltage and membrane wattage or glucose consumption was non-linear (non-ohmic), and it appeared that the resistance of the membrane to current flow was not constant. Based on the electrical formula, resistance = voltage^2/wattage and resistance = voltage/amperage, there was a marked increase membrane resistance when the pH was less than 6.0. The increase in membrane resistance at low pH allowed *S. bovis* to maintain its membrane potential and expend less energy when its ability to ferment glucose was impaired.

Further work will be needed to more clearly define the effect of pH on the resistance of bacterial membranes to proton conductance. Because the the lipid bi-layers are inherently hydrophobic, it is unlikely that protons will pass freely through this portion of the cell membrane. However, as much as 75% of the membrane weight may be protein, and integral proteins must form junctions with the lipid bi-layer. Since pH can affect the ionizable groups on the surface of a protein, it is conceivable that pH could have a direct effect on these junctions.

THE EFFECT OF AMINO ACIDS ON THE GROWTH EFFICIENCY AND HEAT PRODUCTION OF RUMINAL BACTERIA: THE BALANCE OF ANABOLIC AND CATABOLIC RATES

J.B. RUSSELL

Introduction

High-producing ruminants are fed diets which are very rich in protein (as much as 21% crude protein), but there has been considerable controversy regarding the amount of protein which should be degraded in the rumen. *In vitro* and *in vivo* results indicated that amino acids could increase the growth efficiency of ruminal bacteria by as much as 18 and 30%, respectively, but theoretical calculations of bacterial growth indicated that amino acids should have little if any effect on the efficiency of bacterial growth.

Materials and Methods

The ruminal bacterium *Streptococcus bovis* was grown in a purified medium which contained only ammonia as a nitrogen source or amino acids and ammonia as a nitrogen source. Heat production was

monitored with a sensitive microclorimeter, and growth rate was estimated from the increased in optical density. Fermentation end-products were determined by high pressure liquid chromatography, and bacterial protein was assayed by the method of Lowry.

Results and Discussion

S. bovis grew nearly twice as fast (0.9 versus 1.6 h^{-1}) and had a 40% greater growth yield (18 versus 12.5 mg protein/mmol glucose) when amino acids were added to media containing ammonia and glucose, but the glucose consumption rate (88 µmol mg $protein^{-1}h^{-1}$) was unaffected. Calorimetric measurements likewise indicated that amino acid availability had little effect on the catabolic rate (2.1 mW/mg protein), but there was a 40% decrease in the specific heat (j/mg protein). The addition of chloramphenicol to exponentially growing cultures caused a gradual decrease in growth rate and an increase in the specific heat (j/mg protein), and this result indicated that the efficiency of protein synthesis was highly dependent on the anabolic rate.

These growth-rate dependent changes in metabolic efficiency could not be explained by maintenance energy. Amino acids had little effect on the the maintenance energy requirement and maintenance only accounted for a small fraction of the total energy. When growth was inhibited by iodoacetate, an inhibitor of glycolysis, there was a decrease in both the catabolic and anabolic rates, and there was no increase in specific heat. Batch cultures which were forced to grow discontinuously (small doses of glucose only after growth ceased) resumed exponential growth quickly if amino acids were available and the final cell yield was unaffected. When amino acids were not available (only ammonia), the discontinuously fed cultures never grew at the maximum growth rate (always in lag phase), and the yield was 25% lower than cultures receiving one large dose of glucose. These results indicated that the growth efficiency of ruminal bacteria is highly dependent on the balance of anabolic and catabolic rates and that any excess energy may be dissipated as heat.

CELLULOSE DIGESTION BY A GENETICALLY RECONSTRUCTED ENDOGLUCANASE FROM THE RUMINAL BACTERIUM, *BACTEROIDES RUMINICOLA*

G. MAGLIONE, J.B. RUSSELL and D. B. WILSON

Introduction

Cellulose is the most abundant polymer in nature, but mammals do not produce enzymes which can degrade this material. Ruminant animals have developed the capacity for cellulose digestion by exploiting a symbiotic relationship with ruminal microorgansims. The animal provides a habitat for the microbial growth, and the microbes provide fermentation acids that the animal can utilize. The rumen microflora is very complex, but few species of ruminal bacteria are cellulolytic. Recent work has shown that cellulolytic ruminal bacteria are unable to tolerate even modest declines in pH and that there is little cellulose digestion when large amounts of starch are added to the diet .

A variety of noncellulolytic ruminal bacteria are able to utilize cellodextrins, and it has become apparent that some of these bacteria produce endoglucanases which can degrade carboxymethylcellulose. *Bacteroides ruminicola* secretes two immunologically cross reacting CMCases into the culture supernatant. These enzymes also degrade

xylan, but they have very low activity on native cellulose. The lack of cellulose digestion was correlated with the inability of the enzymes to bind tightly to cellulose.

Materials and Methods

The *Bacteroides ruminicola* CMCase was cloned into *E. coli* and the structural gene was sequenced. A piece of DNA corresponding to the cellulose binding domain of a crystalline cellulase was added to the 3+ end of the *B. ruminicola* CMCase. The activity of the reconstructed CMCase on CMC and native cellulose was determined.

Results and Discussion

B. ruminicola produced two CMCases, but both of these enzymes were encoded by the same structural gene. The larger CMCase (88 kd) is encoded in two overlapping reading frames that are -l base out of frame, and the smaller (82 kd) CMCase is encoded in the second reading frame. *B. ruminicola* only produces 88 and 82 kd CMCases but *E. coli* clones containing the 88-kd structural gene also produced a 40.5 kd CMCase. The 40.5 kd CMCase was encoded by the 3' end of the second reading frame, and *E. coli* produced more 40.5 CMCase than 88 or 84-kd CMCase.

The 40.5 kd CMCase (including its regulatory sequences) was joined in frame to the cellulose binding domain of the *Thermomonospora fusca* cellulase E2 structural gene, and this construction took place in 3 steps: 1) a synthetic adaptor (S' ATAAGCTTATGC 3') containing a Hind III site was inserted into the unique SacII site of plasmid pC39 to produce pCBD2, 2) a 414 bp Hind III-Pst I fragment coding for the E2 cellulose binding domain was excised from pCBD2 and ligated into pUC19 (cut at the same restriction sites to produce pCBD3), and 3) a 1160 bp HindIII-HindIII fragment encoding the 40.5 kd CMCase was excised from pC34 and ligated into HindIII cut pCBD3 to produce pGF7. Recombinants carrying the fragment with the correct orientation to produce a fusion protein were identified by the CMC-overlay assay and their structure was confirmed by restriction mapping.

The reconstructed CMCase (RCMCase) was purified, and its specific activity which was 25 fold greater than the crude extract. The final yield was 25%. The purified RCMCase gave a single band on SDS-polyacrylamide gel electrophoresis, and its molecular weight was 54,000 vs the 40,500 found for the CMCase. RCMCase reacted with antibodies which had been directed against both the 40.5 kd CMCase and cellulase E2. The RCMCase bound to Avicel as expected while the CMCase did not. The enzymatic activity of the purified RCMCase was determined on CMC, acid swollen cellulose and ball milled cellulose at several enzyme concentrations, and these results were plotted to determine the amount of enzyme required to give 4% digestion. The RCMCase had only a slightly higher specific activity on CMC than the CMCase, but it was 10 times more active on acid swollen cellulose. The CMCase was never able to hydrolyze 4% of the ball-milled cellulose, but the the RCMCase was able to hydrolyze this substrate at a rate of 1.2 umol/mg/protein/min.

Thermomonospora fusca has an exocellulase (E3) which degrades filter paper at a rapid rate, and this enzyme showed synergism with the RCMCase, but not the CMCase. Neither the CMCase nor the BCMCase was able to digest 4% of the filter paper, but a mixture of the BCMCase and E3 was twice as active as E3 alone. The mixture of the CMCase and E3 had slightly less activity than E3 alone.

These results indicate that it is indeed possible to change the activity of cellulolytic enzymes from ruminal bacteria by the addtion of cellulose binding sites. Since *B. ruminicola* is much more acid resistant than the cellulolytic bacteria which already inhabit the rumen, it may be possible to convert *B.ruminicola* into an acid-resistant ruminal bacteria. Work is currently underway to transfer the RCMCase back to *B. ruminicola*.

ANALYSIS OF THE COMBINED EFFECTS OF pH AND DILUTION RATE ON THE CELLULOSE FERMENTATION OF *Ruminococcus flavefaciens*

Y. SHI and P.J. WEIMER

Introduction

Cellulose is the most abundant component of plant cell wall material, and thus is a major contributor to the energy of the forage-fed ruminant. A more complete understanding of cellulose degradation by ruminal cellulolytic microorganisms requires quantitative information on substrate consumption, product formation, and cell yields under different environmental conditions. These fundamental process parameters are best obtained from studies with pure cultures of cellulolytic microorganisms in continuous culture.

Materials and Methods

R. flavefaciens (one of the most important of the ruminal cellulolytic bacteria) strain FD-1 was grown at 39 C in a modified Dehority's medium with cellulose as growth-limiting nutrient. All experiments were performed in a continuous culture device (working volume 940 mL) in which the cellulose-containing medium was delivered as a CO_2-segmented slurry. For each chemostat run, 5-13 samples were collected at system steady state over a run period of 2-7 days. Under these conditions, the dilution rate (D) is equal to the bacterium's specific growth rate constant m.

Culture samples were analyzed for residual cellulose by a modified neutral detergent method, and culture supernatants were analyzed for soluble sugars (anthrone method), reducing sugars (DNS method), and nongaseous fermentation products (by HPLC). The centrifuged pellets were analyzed for total nitrogen content by the Dumas combustion method, and cell mass was estimated from the nitrogen values using an assumed cell organic matter composition of $C_5H_7O_2N$ and the assumption that cells were 90 % organic material.

Data were collected at steady state from twenty separate chemostat runs at various combinations of pH (6.0-7.1) and D (0.02-0.06 h^{-1}, corresponding to retention times of 50 h - 16.7). The observed data for each fermentation parameter were fit to a response surface equation using the General Limiting Model of SAS, and the relationships of each parameter to pH and D were visualized via contour plots.

Results

Fig.1 shows the response surfaces for the most statistically-significant of the fermentation parameters. Fractional cellulose consumption (Fig.1A; r^2=.781) showed an optimum at low D and near pH 6.4, but the well-spaced contours indicate that cellulose digestion itself changed only slightly with pH except at high D. Indeed, the total rate of cellulose digestion (the product of fractional cellulose consumption, feed cellulose concentration, and D) was strongly affected by D but only weakly affected by pH.

Cell yield (Y) displayed an optimum at higher D values and at pH values near 6.6 (Fig.1B, r^2=.814). Solution of the response surface equations for various values of D at a given pH allowed construction of Pirt plots (1/Y vs. 1/D) for each pH. The slope of these plots is equivalent to the maintenance energy *m*. These predicted values for *m* at pH 6.6, 6.4, 6.2, and 6.0 were 38, 45, 66, and 152 mg cellulose / g cells / h. Thus the decreases in cell yield appear to be due to absolute increases in *m* at low pH, and relative increases in *m* at low D (lower growth rates). The cell yield data, combined with the moderate insensitivity of cellulose hydrolysis to low pH, suggest that poor cellulose digestion at low ruminal pH may be mediated primarily by poor microbial growth yields — which ultimately reduce

the numbers of cellulolytic microorganisms in the rumen.

The concentrations of soluble sugars remained low in all of the fermentations (Fig.1 C, r^2=.570), suggesting that the cellulose hydrolysis was the rate-limiting step in the digestion process, and that cells retained active fermentative capabilities within the pH and D ranges tested. Soluble sugars were almost exclusively glucose, with almost no detectable amounts of cellobiose or cellodextrins; this suggests that uptake of the oligomers was extremely efficient.

Acetate yield (Fig 1D, r^2=.641) was highest at low D, where it was relatively independent of pH. At D > 0.03 h^{-1}, acetate yield decreased as pH was shifted away from the optimum pH for growth. This pattern is similar to that of growth yield, indicating that acetate production (which generates ATP via the acetate kinase reaction) and cell growth are tightly coupled. However, because the response surface model gave a poor fit for succinate yield (r^2 = 0.311), a comparison cannot be made between succinate production and cell yield.

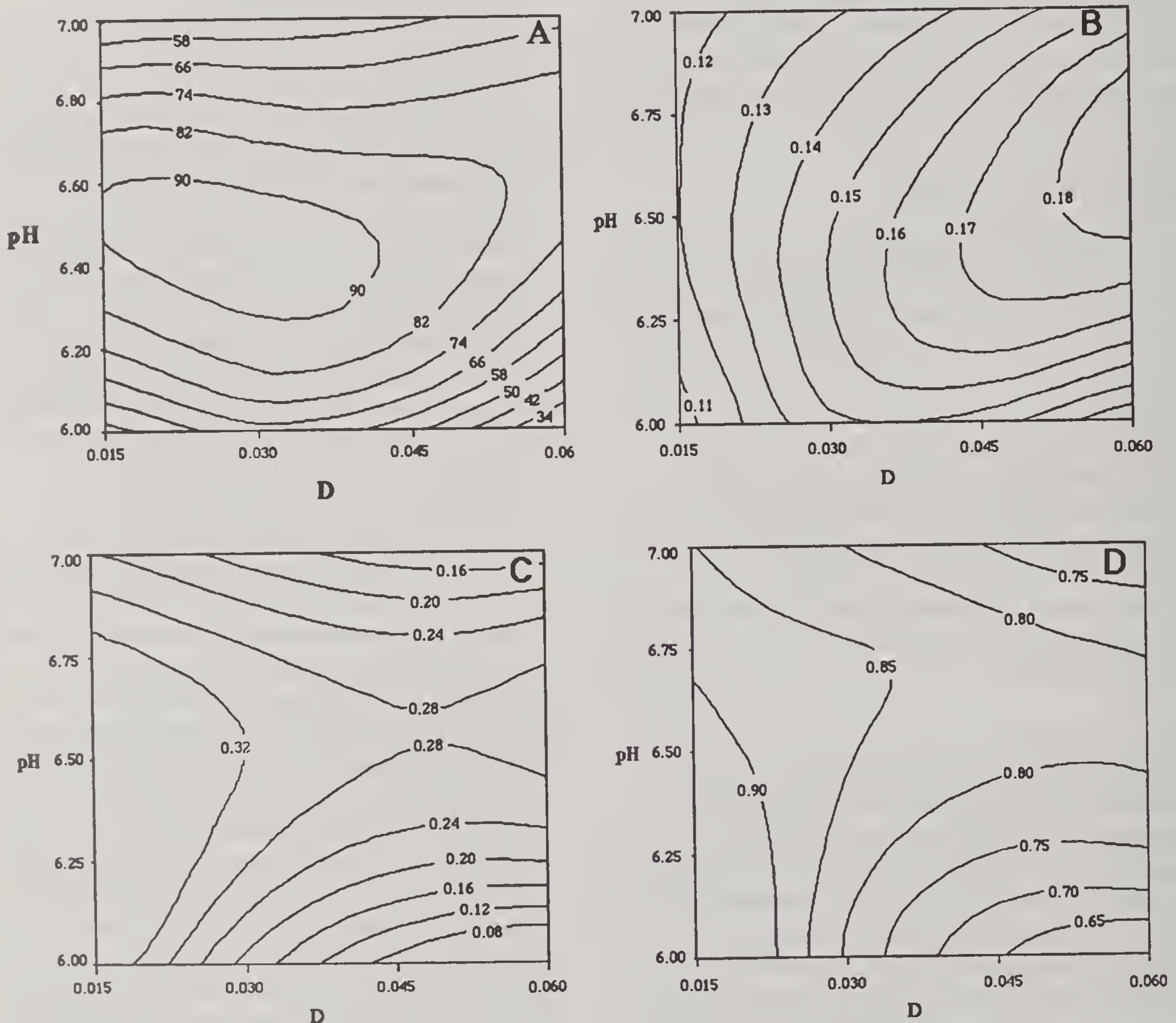


Figure 1. Response surfaces for fermentation parameters of R. flavefaciens FD-1 in cellulose-limited continuous culture. A) Fractional cellulose consumption; B) Cell yield (g D) Acetate yield (mol acetate produced / mol anhydroglucose consumed).

INHIBITION OF THE RUMINAL CELLULOSE FERMENTATION BY EXTRACTS OF CICER MILKVETCH (Astragalus cicer)

P.J. WEIMER, R.D. HATFIELD and D.R. BUXTON

Introduction

Cicer milkvetch is a perennial Russian legume whose winterhardiness and resistance to insect and grazing pressure has attracted interest as a forage and pasture crop in the western U.S. Conflicting reports concerning its digestibility suggest that the forage contains an antinutritive material which may affect ruminal fibrolytic bacteria.

Materials and Methods

Dried and ground samples of cicer milkvetch were obtained from various studies and included the following: ground whole herbage from a two-year, three-cut study of varieties Monarch and Lutana, conducted near Ames, IA in 1984-5; ground leaf and stem material grown from the same batch of seeds in the USDFRC greenhouse in 1991; and ground leaf and stem material from a 1991 study of cultivars selected for palatability to sheep (supplied by N. Ehlke, U. of Minnesota).

In vitro fermentations were conducted in serum vials containing prereduced media under CO_2 (McDougall buffer for mixed rumen microflora, modified Dehority medium for pure cultures of *Ruminococcus flavefaciens* FD-1or *Fibrobacter succinogenes* S85). Sigmacell 50 cellulose (1% w/v) was used as energy source. Experiments with cellobiose as energy source were conducted in anaerobic crimp-seal tubes under similar conditions. The mixed ruminal inocula were obtained from an alfalfa-fed nonlactating Jersey cow. Residual cellulose was determined by a modified neutral detergent method.

Cicer milkvetch extracts were prepared by incubating 1 g of dried ground material in 10 mL of water at 39 C for 3 h. The resulting suspension was squeezed through 30 mm nylon sheets, then gassed under N_2 and either used immediately or stored overnight at 5 C.

Results and Discussion

Comparison of plant material collected from the same set of replicated field plots revealed that the digestion of NDF in Monarch cicer milkvetch was much poorer than that of Apollo II alfalfa but was improved considerably by extracting the cicer with warm water. This extract showed a transient (16-24 h) and concentration-dependent inhibition of cellulose digestion by mixed ruminal microflora, but a permanent inhibition of cellulose digestion by the pure cultures of cellulolytic bacteria. However, little or no inhibition of cellobiose fermentation by these same pure strains was observed (Table 1), suggesting that the inhibitor specifically affected a step in the cellulose depolymerization process. The adherence of cells to cellulose, normally a required and distinguishing feature of the ruminal cellulose fermentation, was also transiently inhibited in the mixed culture but permanently inhibited in the pure culture. This fact, coupled with the failure of the extracts to inhibit the cellulase complex of the aerobic fungus *Trichoderma reesei*, suggests that the inhibitor may act by preventing adhesion of the fibrolytic bacteria to cellulose.

The inhibitory activity was strongest in extracts prepared from regrowth material of field-grown plants and was localized in the leaves but not in the stems (Table 2). Interestingly, only weak inhibition was observed in greenhouse-grown material from the same seed batch used to generate the field-grown material in Ames, suggesting a strong environmental component in production of the inhibitor (perhaps related to root nodulation, as the greenhouse-grown material lacked nodules). Extracts from three cultivars selected in the Minnesota study for high

palatability to sheep gave greater inhibition than did extracts from three cultivars selected for low palatability. The data suggest that selection based on palatability may result in a plant with lower digestibility under certain conditions (e.g., high rate of passage). However, the transient nature of the inhibition of mixed ruminal microflora suggests that the inhibitor is metabolized by some members of the rumen microbial population.

Table 1. Effect of water extract (10% w/v) of Monarch cicer milkvetch (ground whole herbage) on cellulose degradation and on fermentative growth on cellobiose by pure cultures of ruminal cellulolytic bacteria.

Bacterium	Cicer extract	Cellulose degradation after 18 h incubation	Growth (A_{600}) of culture on cellobiose after 18 h
Ruminococcus flavefaciens FD-1	——	35.5 ± 0.1	0.628 ± 0.013
	(4% v/v)[a]	< 0.1	0.647 ± 0.006
Fibrobacter succinogenes S85	——	35.4 ± 0.1	1.07 ± 0.04
	(4% v/v)[a]	< 0.1	0.627 ± 0.050

[a] equivalent to material released from 4 g of cicer milkvetch per liter of culture medium.

Table 2. Fermentation of cellulose by mixed ruminal microflora treated with cicer milkvetch extracts derived from different plant parts.

Cicer material		mg cellulose degraded at different extract concentrations			
		Leaf extract		Stem extract	
Cultivar[a]	Source[b]	1[c]	3[c]	1[c]	3[c]
Monarch	G Immature	44.3 [z]	31.1 [z]	39.7 [y]	47.4 [z]
	G Mature	43.8 [z]	18.7 [y]	42.8 [y]	43.0 [y]
L2-17-34D	F	19.4 [x]	0. 1[x]	39.2 [y]	42.7 [y]
L7-18-G56	F	20.5 [x]	0 [x]	35.5 [y]	34.1 [y]
L14-11-F39	F	32.9 [y]	1.1 [x]	34.7 [y]	37.9 [y]
H6-2-65D	F	5.9 [w]	0.6 [x]	42.1 [y]	41.8 [y]
H14-7-65G	F	1.6 [w]	0.2 [x]	42.4 [y]	18.8 [x]
H16-1-66	F	9.1 [w]	0.8 [x]	42.8 [y]	41.5 [y]
Control (no extract)		-38.1[y]-			

[a] L and H = cultivars selected by N. Ehlke for low and high palatability, respectively, to sheep.
[b] G= greenhouse grown in Madison in 1991; leaf maturity from plant material harvested on the same date. F= field grown near St. Paul in 1991.
[c] 1 and 3% v/v of an extract prepared as 1 g dry plant material/10 ml water, equivalent to 1g and 3g, respectively, of plant material per liter culture volume. Values in same column having different superscripts differ ($p < .05$).

CHARACTERIZATION OF THE DEGRADATION PATTERN OF ALFALFA PECTINS BY RUMEN MICROBES

R.D. HATFIELD and P.J. WEIMER

Introduction

Alfalfa has many advantages over other forage crops utilized by the dairy industry and by other ruminant-based enterprises. Among these are the high protein levels found in the leaves even at relatively mature stages of development. The full potential benefits of this high protein content are not realized due to high degradation rates and conversion to ammonia in the rumen, leading to increased nitrogen excretion. To improve the efficient utilization of alfalfa, the rapidly degraded protein should be matched with an equally rapidly-degraded carbohydrate source such that energy would not be limiting to rumen microbes, thus assuring that the plant protein would be more efficiently converted to microbial biomass (including protein) which would benefit the ruminant. Pectic polysaccharides represent a potential source of rapidly degradable carbohydrate. A significant portion of the total structural polysaccharides in alfalfa cell walls is pectin, with stems ranging from 10 to 20% while leaves contain 25-26%. This work was undertaken to investigate the degradation characteristics of alfalfa pectins by rumen microbes.

Methods

Two rhamnogalacturonan fractions were isolated from alfalfa leaves by chemical extraction (hot buffer solublization and ammonium oxalate extraction) and subjected to *in vitro* fermentation by mixed ruminal microflora from an alfalfa fed cow. Inoculation times were 0,1,2,4,6,8,12, and 24h. The supernatants after each samples incubation were analyzed for total sugars, total uronics, and neutral sugar composition. A second set of experiments involved characterization of pectin degradation from isolated alfalfa cell walls. Walls were isolated from leaves of immature plants and stems of plants at full bloom. Individual samples were inoculated with rumen fluid and incubated for 0,2,4,6,8,12, 24,36,48, and 96h. After the appropriate incubation period, samples were made 80% ethanol and stored at -20°C until removed for analysis. Insoluble material was pelleted by centrifugation, the supernatant removed, and the pellet freeze dried. Samples were suspended in phosphate buffer (pH 6.9), heated for 2h at 80-90°C cooled to 55°C and α-amylase and amyloglucosidase added to remove starch. After a 2.5h incubation, samples were made 80% ethanol, insoluble material pelleted, and freeze dried. Individual samples were analyzed for total sugars, total uronics, and neutral sugar composition.

Results and Discussion

The two pectic fractions isolated from alfalfa leaves were similar in chemical composition. Each was composed of rhamnogalacturonans with a high degree of arabinose and galactose substitution. It was thought that, due to the complex substitution patterns on these pectic polysaccharides, degradation by rumen microbes may be slowed or restricted. However, degradation of both samples by rumen cultures was rapid having a half life of approximately 4 hours and being completely degraded after a 12 hour incubation (Fig. 1A). There were no differences in the rate of polygalacturonic acid degradation as compared to the neutral sugar residues in these fractions. The rate of degradation of individual neutral sugar residues (Ara, Gal, Rha) was the same as that observed for the combined total sugar measurement (Fig. 1A). The complex linkage patterns found in these samples did not limit degradation by the microbes. In addition, the rapid degradation of the alfalfa fractions did not result in a pH decline and the VFA production profile was shifted to increased acetate and decreased butyrate.

For comparison, citrus pectin, galacturonic acid, and arabinogalactan were also degraded by rumen microbes (Fig. 1B).

Although the arabinogalactan from larchwood had a similar neutral sugar composition as that found in the alfalfa fractions, its degradation rate was more restricted suggesting that there may be linkage patterns that are harder for rumen microbes to degrade. This decrease in rate for arabinogalactan was not due to solubility as it was quite soluble in the rumen fluid-buffer system.

Initial degradation experiments using 1mm ground samples prepared from stem and leaf tissues indicated that pectic polysaccharides contained within the cell wall matrices were degraded by rumen microbes at approximately the same rate as the isolated forms (Fig.1C & 1D). There were only minor differences between the two plant tissues with a slightly slower rate of pectic polysaccharide degradation in the stem samples. In stem samples recovered after 24h incubation and subjected to cell wall fractionation scheme, only 3-4% of the original pectic material could be recovered. Degradation of the pectic fraction is nearly 100% even from tissues that are highly lignified. The plateau in the total uronics (Fig. 1D) reflects the glucuronic acid content of the alfalfa cell walls and parallels the degradation pattern of the xylans (xyl Fig. 1C).

These findings would indicate that pectic polysaccharides represent a potential energy source that has a rapid degradation in the rumen. Alfalfa plants with higher total pectic polysaccharides should provide a greater amount of easily utilized energy and may provide additional energy to better utilize the rapidly degraded protein.

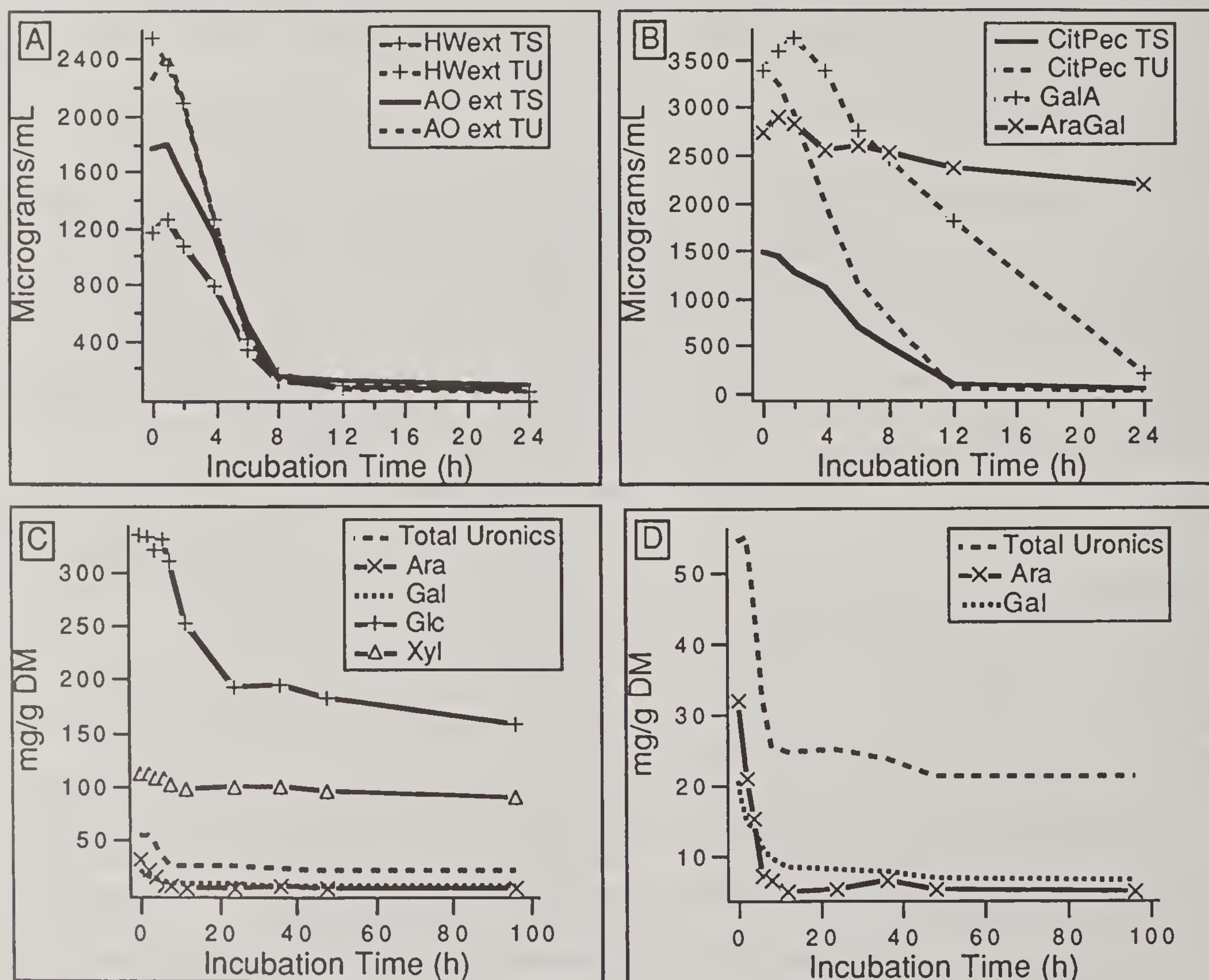


Figure 1. Degradation patterns of isolated alfalfa pectins, commercial polysaccharides, and intact cell wall materials. A) HWext=hot buffer extract of alfalfa cell walls, AOext=ammonium oxalate extract of alfalfa walls; B) CitPec=citrus pectin (Sigma), GalA=galacturonic acid, AraGal=arabinogalactan from larchwood; C) Degradation of pattern of individual sugars from alfalfa stem cell walls; D) Enlargement of lower portion of C. For all graphs TS=total neutral sugars and TU=total uronics.

EFFECT OF MATURITY OF ENSILED ALFALFA ON MILK PRODUCTION OF MID-LACTATION COWS

C.F. LEE and L.D. SATTER

Introduction

This is the continuation of a large study of the effect of alfalfa maturity on milk production. It has long been known that increased maturity reduces forage quality, but the extent to which maturity affects milk production needs to be quantitatively determined.

Procedures and Methods

Alfalfa was harvested under a 3-cut or a 4-cut system in 1989, 1990 and 1991 and ensiled. Four-cut alfalfa had higher quality but yielded less than 3-cut alfalfa, especially in the last year. Four-cut alfalfa had higher crude protein, lower fiber, and lower lignin contents (Table 1). More frequent cutting reduced stand persistence of 4-cut alfalfa.

Experiment 1. Forty-eight mid-lactation cows were randomly assigned to a 2 x 2 factorial trial with a switchback design. Treatment factors were two forage levels in the diets (55 and 75%), and two maturities of alfalfa silage (3-cut and 4-cut). Diets contained alfalfa silage, high moisture corn, roasted soybeans, soybean meal, mineral and vitamin supplement. The experiment contained a 2 week pretrial period, and two experimental periods of 4 weeks each. Cows within a forage level were switched at the end of the first 4 week period.

Experiment 2. Forty-eight mid-lactation cows were randomly divided into a 2 x 2 factorial trial. Diet treatments were similar to the first trial except that roasted soybeans were increased 1.5 to 2.0% in the 3-cut alfalfa diets to balance the undegradable protein (UIP) concentration in both diets. The experiment contained a 2 week pretrial, a 7 week treatment period, followed by a 2 week switchback period when diets within a forage level were switched.

Results

Experiment 1. Diets with 3-cut alfalfa silage had higher fiber and lower protein content than 4-cut diets (Table 2). Figure 1 shows the weekly milk production changes through the whole experiment. When the two maturities of alfalfa were switched (S1) between the two groups fed the same forage levels, milk production responded immediately. The 4-cut alfalfa supported higher milk production in both the first treatment period and in the switchback period. Milk production of 55% forage groups was significantly higher than the 75% forage groups (P = 0.008). Four-cut alfalfa silage supported higher milk production than 3-cut alfalfa silage (P = 0.099). Milk fat and milk protein percent were not affected by treatment factors.

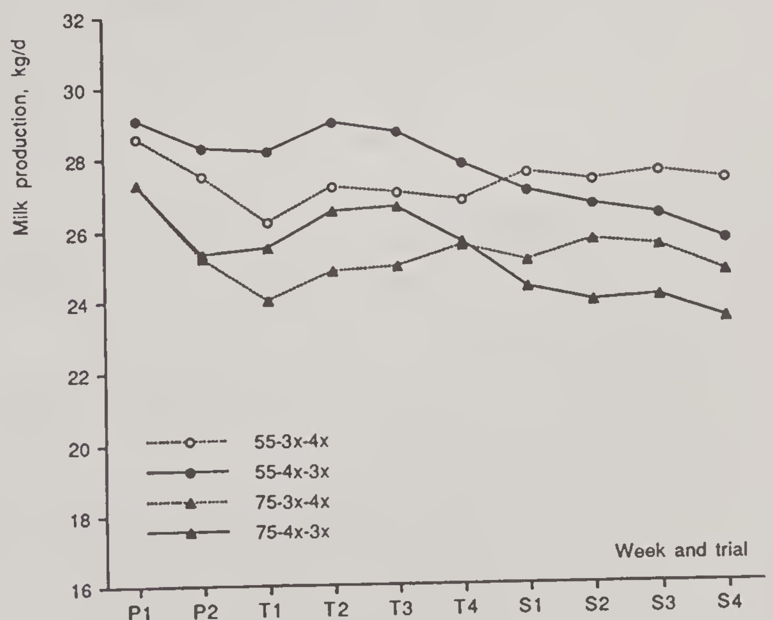


Figure 1. Milk production of mid-lactation cows fed 3-cut or 4-cut alfalfa silages in diets containing 55 or 75% alfalfa silage (DM basis) (Trial 1).

Experiment 2. Figure 2 shows the weekly milk production of the four groups. Cows fed 4-cut alfalfa produced more milk than cows fed 3-cut alfalfa. Again, when cows were switched (S1) from one forage maturity to another within forage level, milk production immediately responded. Both forage level and maturity significantly affected milk production (P = 0.004, 0.0003). Four-cut alfalfa silage provided significantly higher milk production than the 3-cut alfalfa at both the 55 and 75% forage diets (P = 0.016, P = 0.004). The increased dietary UIP content did not mask any maturity effect. There was no significant difference in milk production between the 75%-4 cut group and the 55%-4 cut group.

Conclusions

Results of these trials generally agree with trials conducted earlier, and it is concluded that each day of delay in alfalfa harvest between early bud to early flower results in about .1 kg decrease in daily milk production for mid lactation cows.

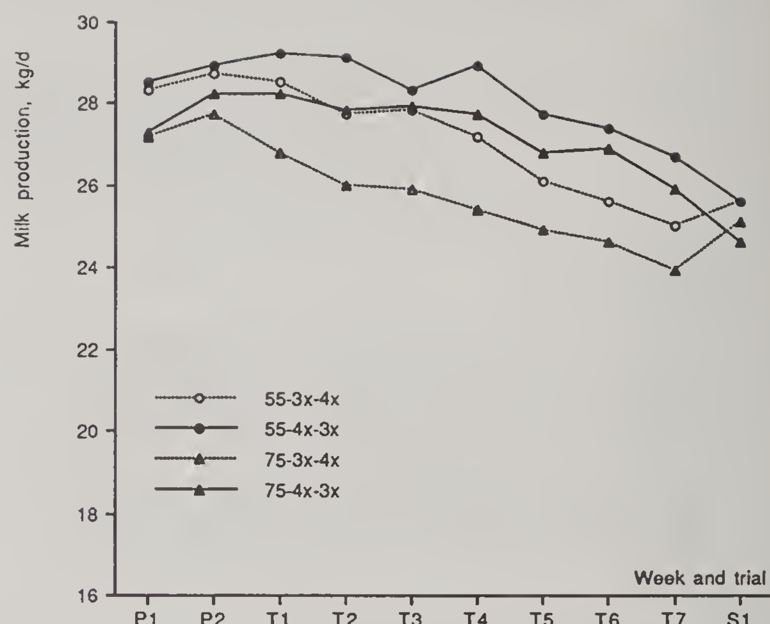


Figure 2. Milk production of mid-lactation cows fed 3-cut or 4-cut alfalfa silages in diets containing 55 or 75% alfalfa silage (DM basis) (Trial 2).

Table 1. Yield and composition of 3-cut and 4-cut alfalfa before ensiling from 1989 to 1991.

Year	DM yield (Ton/A)	MSW	CP	NDF	ADF	ADL
			% DM basis			
1989						
3-cut	3.9	4.0	17.0	44.5	35.4	6.77
4-cut	2.9	2.7	19.8	39.1	30.0	5.31
1990						
3-cut	4.4	-	18.6	45.4	37.0	7.08
4-cut	4.2	-	20.8	39.6	31.2	5.54
1991						
3-cut	3.2	3.7	17.3	44.3	35.7	6.9.0
4-cut	2.8	2.6	19.8	39.3	31.5	5.6.0

MSW, mean stage by weight; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin.

Table 2. Diet composition (Trial 1 and 2, DM basis, %).

Item	55%-3x	55%-4x	75%-3x	75%-4x
Ingredients				
Alfalfa silage	55.0		75.0	
High moisture ear corn	28.0*		13.2*	
Roasted soybean	10.0*		10.0*	
Soybean meal	4.6		—	
Vit & Mineral	2.4		1.8	
Chem. composition of diet				
NDF	37.5	33.9	41.7	38.1
ADF	29.8	25.9	33.9	30.4
CP	20.7	21.3	19.9	21.7

*For Trial 2, high moisture ear corn values were 26.5 and 28.0 for 55%-3x and 55%-4x, and 11.2 and 13.2 for 75%-3x and 75%-4x. Comparable values for roasted soybeans were 11.5 and 10.0; 12.0 and 10.0.

PROTEIN IS THE FIRST LIMITING NUTRIENT FOR LACTATING COWS FED LARGE AMOUNTS OF HIGH QUALITY ALFALFA SILAGE

T.R. DHIMAN and L.D. SATTER

Introduction

Post-ruminal infusion of protein in lactating dairy cows fed high alfalfa silage diets has resulted in substantial increases in milk yield, but infusion of an energy source has generally had little or no effect. The objective of the present experiment was to determine the response to supplementation of dietary sources of protein or energy on milk yield and composition in cows fed large amounts of high quality alfalfa silage.

Materials and Methods

Forty multiparous Holstein cows were assigned to 5 treatments at parturition. During the first 3 weeks of lactation, cows in all the treatments were fed diets containing forage and grain in a 50:50 ratio. During the experimental period (wk 4-15 of lactation) cows were fed diets containing 75% alfalfa silage supplemented with (1) high moisture ear corn (Ctl); (2) protein from fish meal and blood meal (Prot); (3) fat from prilled animal fat (Fat); (4) protein and fat together (Prot+fat); and (5) protein and glucose infused into the abomasum (Prot+glu). The ingredient and chemical composition of the diets is given in Table 1. Daily feed intake and milk yield were recorded. Milk samples from two consecutive milkings (Sunday p.m. and Monday a.m.) were analyzed for protein and fat content. Body weights were taken once weekly. Weekly blood samples were analyzed for glucose and beta-hydroxybutyrate. Milk fatty acid profile was determined in milk samples collected during wk 5, 7, 9, 11, 13, and 15 of lactation.

Results and Discussion

Supplementation of protein significantly increased dry matter intake (DMI); however, DMI was reduced (12%) compared to control when glucose was infused into the abomasum of cows fed a diet supplemented with protein (Table 2). Fat corrected milk yield was significantly increased when high alfalfa silage diets were supplemented with protein or protein plus fat. Supplementation of fat alone resulted in a much smaller increase in milk production. Infusion of glucose decreased the fat content in milk and thereby reduced daily FCM yield in the Prot+glu group. Milk protein content did not differ among treatments; however there

was a tendency for cows fed fat or infused with glucose to have lower milk protein content. Cows in the Prot+fat group had higher BW gain compared to fat alone. Infusion of glucose in cows fed diets supplemented with protein increased the concentration of glucose in blood plasma. Cows in Ctl, Prot, Fat, and Prot+fat groups had a higher concentration of plasma beta-hydroxybutyrate immediately after the start of treatment compared to the Prot+glu group. Supplementation of fat decreased the proportion of short chain fatty acids (C4-C14:0) and increased the long chain fatty acids in milk fat.

Conclusions

Response to protein supplements and relative lack of response to energy supplements suggest that it was protein, and not energy, that was first limiting milk production in cows fed large amounts of high quality alfalfa silage.

Table 1. Ingredient and chemical composition of diets.

Ingredient	Pretrial P	1 Ctl	2 Prot	3 Fat	4 Prot +fat	5 Prot +glu
	(% dry basis unless specified)					
Alfalfa silage	48.2	75.0	75.0	75.0	75.0	75.0
High moisture ear corn	34.0	23.2	15.1	18.2	9.7	9.7
Roasted soybeans	16.0	-	-	-	-	-
Fish meal	-	-	6.0	-	6.0	6.0
Blood meal	-	-	2.1	-	2.5	2.5
Fat	-	-	-	5.0	5.0	-
Glucose (infused into abomasum)	-	-	-	-	-	1.6 kg/d
NE_L Mcal/kg DM	1.6	1.4	1.4	1.6	1.6	1.6
CP	20.0	18.7	23.8	18.2	23.6	23.6
UIP, % CP	37.9	26.6	37.7	25.9	38.1	38.1

Diets were balanced with minerals and vitamins.

Table 2. Dry matter intake, milk yield and composition.

Item	Ctl	Prot	Fat	Prot +fat	Prot +glu	SEM[1]
DMI, kg/d	22.0[b]	25.4[a]	22.5[b]	23.7[ab]	19.3[c]	2.3
Milk yield, kg/d	29.6[c]	35.7[a]	31.0[bc]	33.5[ab]	35.4[a]	1.05
3.5% FCM, kg/d	28.8[c]	34.1[a]	30.7[bc]	32.6[ab]	31.1[bc]	1.01
Milk composition, %						
Fat	3.37[a]	3.24[a]	3.44[a]	3.36[a]	2.78[b]	.09
Protein	2.92	2.93	2.85	2.95	2.86	.05

[abc]Means with different superscripts differ ($P < .05$).
[1]Standard error of means.

DEGRADABLE INTAKE PROTEIN AND ITS EFFECT ON MICROBIAL PROTEIN SYNTHESIS

T.R. DHIMAN and L.D. SATTER

Introduction

Hoover and Stokes (1991) reported that total carbohydrate digestion in the rumen was not affected by the proportion of nonstructural carbohydrate and degradable intake protein (DIP); however, microbial efficiency (g microbial N per kg carbohydrate digested) increased linearly as DIP content of the diet increased. The objective of the present experiment was to determine the effect of increasing amounts of rumen degradable protein on microbial protein synthesis using branched chain amino acids as an indicator of protein flow to the intestine.

Materials and Methods

Five mid-lactaction cows fitted with rumen cannualae were randomly assigned to 5x5 Latin square design experiment. Each period was 17 days. Within each period there were subperiods: 1, 2, and 3 of 5, 5, and 7 days, respectively. Cows were fed a diet containing (dry basis) 20% alfalfa silage, 40% corn silage, 30% dry shelled ground corn, 8% soybean meal, and minerals and vitamins. Diets were fed as a TMR twice daily. The chemical composition of feed ingredients is given in Table 1. In addition to the basal diet, cows were given casein infusions at five different levels: 0 (Trt 1), 500 (Trt 2), 1000 (Trt 3), 1500 (Trt 4), and 2000 (Trt 5) g/d of casein into the abomasum or rumen. During subperiod 1 in each period, casein was infused into the abomasum (A100); in subperiod 2, 10% of each casein level was infused into the abomasum (A10) and during subperiod 3 the full amount of casein was infused into the rumen (R100). A 10% solution of sodium caseinate was prepared daily and infused continuously (except for the time cows spent in the milking parlor (1/2 h per day). Daily feed intake and milk yield were recorded. During the last 2 days of each subperiod milk samples were collected from 4 consecutive milkings and composite samples of the a.m. and p.m. milkings were analyzed for composition. Blood samples were collected during the last 2 days of each subperiod at 5 h post feeding from the tail vein or artery. Deproteinized blood plasma samples were analyzed for glucose, urea, and amino acids. Concentrations of ammonia and amino acids were determined in rumen liquor samples collected during the last day of each subperiod at 5 h post feeding.

Results and Discussion

Dry matter intake was decreased at the highest level of casein infusion (Table 2). Level of casein infusion had no effect on milk yield, milk composition or blood plasma glucose concentration. Urea concentration in blood plasma and NPN in milk were increased with increasing level of casein infusion into the abomasum and rumen (A100 and R100) (Fig. 1). Concentration of rumen ammonia increased linearly with increasing level of casein infusion into the rumen; however, the concentration of free amino acids in rumen fluid did not change. The concentration of individual and total branched chain amino acids (isoleucine+leucine+ valine) (BCAA) increased linearly with increasing level of casein infusion into the abomasum (A100). However, when the same amount of casein was infused into the rumen, there was no change in BCAA concentration (Fig. 1). Casein infusion into the abomasum at a rate equal to 10% of the rumen infusion resulted in an increase in BCAA concentration at highest level of casein infusion.

Conclusions

Results suggest that increasing the supply of rumen degradable protein in the rumen with the diet used in this study did not increase microbial protein synthesis and, in turn, had no effect on protein supply to the animal.

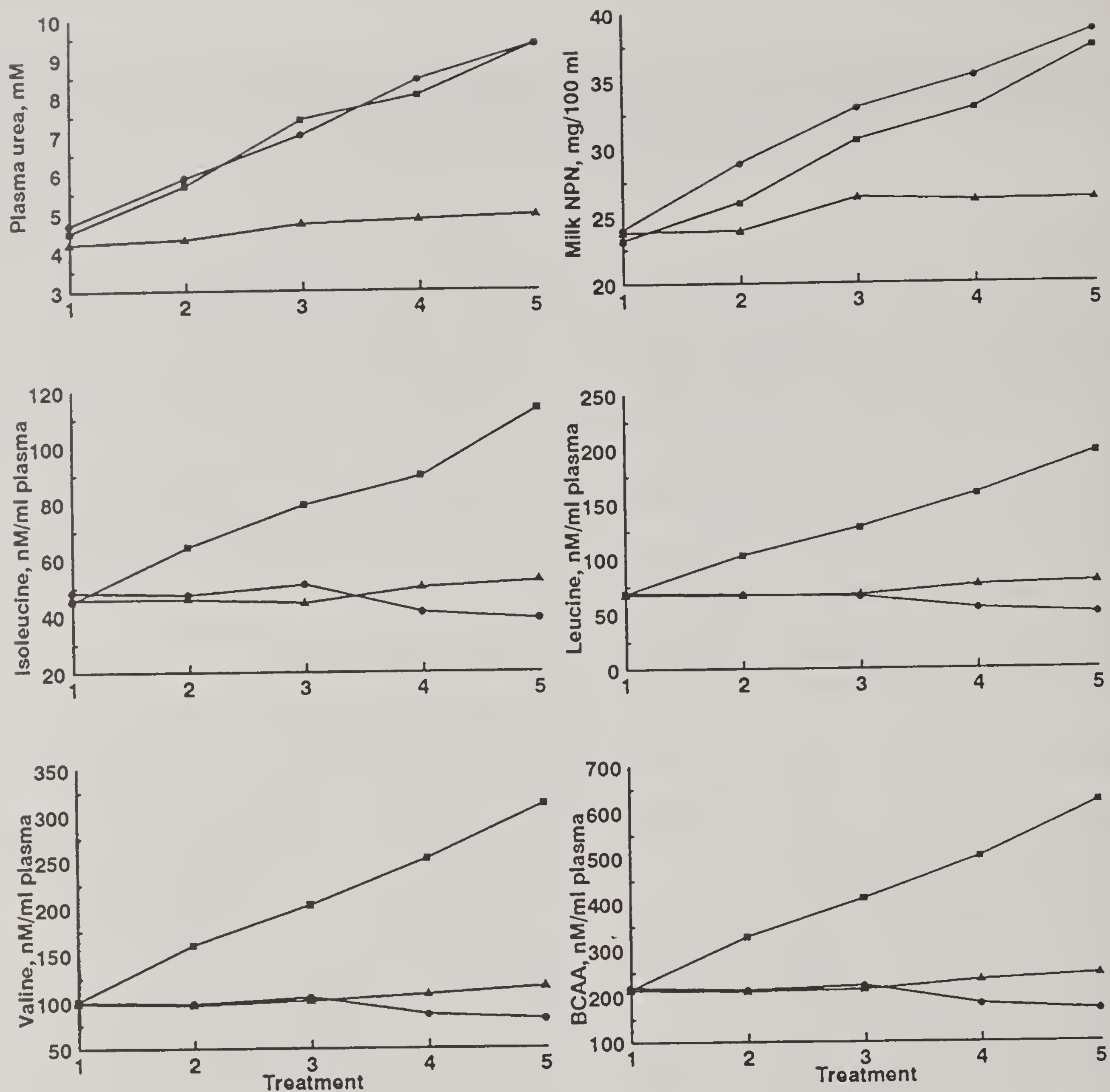


Figure 1. Effect of rumen degradable protein on concentration of urea in blood plasma, NPN in milk and branched chain amino acids (BCAA) in blood plasma.
BACC = (Isoleucine+leucine+valine). *A100* ■ *A10* ▲ *R100* ●

A100 = casein infusion into the abomasum; 0, 500, 1000, 1500, and 2000 g/d in treatment 1, 2, 3, 4, and 5, respectively.
A10 = casein infusion into the abomasum; 0, 50 , 100, 150, and 200 g/d in treatment 1, 2, 3, 4, and 5, respectively.
R100 = casein infusion into the rumen; 0, 500, 1000, 1500, and 2000 g/d in treatment 1, 2, 3, 4, and 5, respectively.

Table 1. Chemical composition of feed ingredients.

Ingredient	DM	CP	NDF	ADF	UIP[1]
		% dry basis			% of CP
Alfalfa silage	50.5	20.4	38.7	32.5	23
Corn silage	35.8	8.5	43.9	23.1	31
Corn	90.2	9.3	—	—	52
Soybean meal	90.4	46.9	—	—	35

[1]Estimates of UIP were NRC (1989).

Table 2. Nutrient intake

Parameter	Treatment 1	2	3	4	5	SEM
DM intake, kg/d	23.5[a]	22.9[ab]	23.9[a]	22.0[ab]	21.2[b]	.6
DIP[1], kg/d	2.17[ab]	2.10[ab]	2.21[a]	2.04[ab]	1.99[b]	.07
UIP[2], kg/d	1.11[ab]	1.08[ab]	1.14[a]	1.05[ab]	1.02[b]	.03

[abc] Means in the same row with different superscript differ P<.05.
[1]Rumen degradable intake protein.
[2]Undegradable intake protein.
[1,2]Does not include protein cows received from the infusion.

UREA VERSUS TRUE PROTEIN FOR LACTATING DAIRY COWS FED FORAGE AS MIXTURES OF ALFALFA AND CORN SILAGES

G. A. BRODERICK, W. M. CRAIG and D. B. RICKER

Introduction

Typical dairy rations formulated from alfalfa and corn silages contain substantial amounts of NPN. When these forages are fed, milk production might be stimulated by supplementing with true proteins which were resistant to rumen degradation. Merchen and Satter (J. Dairy Sci. 66:789, 1983) reported that the amount of nonammonia-N apparently digested in the small intestine, as a proportion of N-intake, was increased 35% when cows were fed alfalfa silage containing 66% DM versus alfalfa silage with 29 or 40% DM. Drier haylage may undergo extensive heating in the silo, causing protein to become resistant to rumen degradation. Therefore, response to feeding resistant proteins could vary with DM content of alfalfa silage. Two studies were conducted with lactating cows fed diets in which alfalfa and corn silages each made up about half of the dietary forage. The objectives of these trials were to determine: 1) if there were differences due to form of dietary N supplements (Trial 1), and 2) whether production response to NPN or true protein differed when alfalfa silage was ensiled with low DM (LDM) or high DM (HDM) contents (Trial 2).

Materials and Methods

Trial 1 was conducted with second-crop alfalfa silage averaging 60% DM, with (DM basis) 19.8% CP and 49% NDF, and (total N basis) 7.7% ADIN. This haylage was fed with corn silage at, respectively, 26 and 30% of ration DM (Table 1). Twelve cows in early lactation were fed these forages plus concentrate containing one of the four N supplements in a 4X4 Latin square (a switchback design where each cow eventually gets each diet over the course of the trial; Table 1): urea, solvent soybean meal (SBM), meat and bone meal (MBM), or a mixture of SBM plus MBM. Supplements supplied 24-29% of total dietary CP. Cows were fed their respective diets for 3-week periods before switching to the next diet (total 12 weeks). Milk production data were from the last two weeks of each period. In trial 2, third-crop alfalfa was cut and wilted to either 35-40% DM (LDM) or 55-60% DM (HDM) and ensiled into two upright concrete stave silos. The LDM haylage averaged 39% DM, with (DM basis) 19.9% CP and 49% NDF, and (total N basis) 9.1% ADIN and 53% NPN; HDM haylage averaged 59% DM, with (DM basis) 19.4% CP and 48% NDF, and (total N basis) 9.8%

ADIN and 41% NPN. These haylages were fed with corn silage as 26 to 28% of ration DM (Table 1). Sixteen early lactation cows were fed four diets in a second 4X4 Latin square (Table 1): LDM or HDM haylage supplemented with either urea or SBM plus MBM. Extents of net rumen protein escape estimated in vitro for SBM and MBM were 27 and 53%. Supplemental N sources provided 28-32% of dietary CP. Experimental design was the same as in Trial 1.

Results and Discussion

In Trial 1, feeding MBM resulted in a trend ($P < .07$) for reduced DM intake when expressed as a percent of BW (Table 2). However, BW gain was increased with feeding of MBM, with gain on SBM and SBM plus MBM being intermediate. Feeding MBM depressed ($P < .03$) total and true protein content of milk; protein supplement had no other significant effects on any other milk production trait. These data indicated that, in cows fed half of their forage as 60% DM alfalfa silage and producing only 32-33 kg/day, NPN supplement was as effective as true protein from degradable (SBM) or resistant (MBM) sources.

In Trial 2, supplementing LDM haylage with urea resulted in about 2 kg/day lower DM intake ($P < .001$); BW change was less positive on both LDM and HDM haylage when urea was fed (Table 2). When LDM haylage was fed, supplementing with true protein (SBM plus MBM) increased production of milk and milk components; milk and milk protein yield were elevated 3.0 and .07 kg/day. The effect of true protein was not as great on HDM haylage: milk and milk protein yield were increased only 1.0 and .04 kg/day. Efficiency of milk production was greater ($P < .001$) with LDM than HDM haylage. Blood and milk urea concentrations were similar within diet; both were substantially lower ($P < .001$) with feeding true protein (SBM plus MBM) versus urea. Greater intake of dietary NPN on LDM haylage diets appeared not to influence milk or blood urea levels. Production was clearly greater with true protein supplementation in this trial.

Summary and Conclusion

At low levels of production (33 kg milk/day) and relatively high rates of intake (DM equal to about 3.8% of BW), form of supplemental CP did not influence milk yield in cows fed 25% of dietary DM from high DM haylage. Compared to NPN, true protein supplementation substantially improved yield of milk and milk components in cows fed low DM haylage; however, yield response to true protein was much reduced in cows fed high DM haylage. It may be speculated that cows fed low DM alfalfa silage, which contains more NPN, might already have sufficient rumen degradable N and thus make little use of additional NPN. Cows fed high DM alfalfa silage, which contains less NPN, might require additional rumen degradable N; therefore, production response to NPN or true protein might be similar on relatively high energy diets formulated from alfalfa and corn silage. True protein supplements, particularly those resistant to rumen degradation, will be used to greatest advantage on high alfalfa silage diets containing high basal levels of NPN.

Table 1. Ration composition (percent, DM basis).

Trial	Ingredient	Diet			
1		Urea	SBM	MBM	SBM + MBM
	Haylage	25.5	25.5	25.5	25.5
	Corn silage	30.1	30.1	30.1	30.1
	Urea	1.33	—	—	—
	SBM	—	9.3	—	4.7
	MBM	—	—	8.2	4.1
	Corn grain	40.0	32.3	35.6	34.1
	Rock phosphate	2.22	2.22	—	1.15
	TMS	.44	.44	.44	.44
	Vit. ADE Conc.	.04	.04	.04	.04
	Sodium sulfate	.27	—	—	—
	Composition				
	CP	15.4	15.4	15.7	15.6
	NDF	30	30	31	31
	ADF	20	21	21	21
	NE_L (Mcal)	1.62	1.65	1.67	1.66
2		39% DM + Urea	39% DM + SBM & MBM	59% DM + Urea	59% DM + SBM & MBM
	LDM Haylage	25.7	25.8		
	HDM Haylage			27.2	27.2
	Corn silage	27.5	27.6	26.9	27.0
	Urea	1.64	—	1.61	—
	SBM	—	5.5	—	5.4
	MBM	—	5.1	—	5.0
	Corn grain	43.0	35.5	42.2	34.9
	Rock phosphate	1.31	—	1.29	—
	TMS	.51	.51	.50	.50
	Vit. ADE Conc.	.05	.05	.05	.05
	Sodium sulfate	.33	—	.32	—
	Composition				
	CP	16.4	16.3	16.3	16.2
	NDF	30	31	30	31
	ADF	18	19	19	20
	NE_L (Mcal)	1.63	1.68	1.63	1.67

SBM = solvent soybean meal; MBM = Meat and bone meal; TMS = Trace minerial salt; LDM = Low DM; HDM = High DM.

Table 2. Intake, BW gain and milk production.

Trial	Trait	Units	Diet				Trt effect
1			Urea	SBM	MBM	SBM + MBM	
	DMI	(kg/d)	25.4	25.3	24.7	24.9	.486
	DMI	(%BW)	3.96[a]	3.89[ab]	3.78[b]	3.82[b]	.065
	BW gain	(kg/d)	.33[b]	.53[ab]	1.02[a]	.79[ab]	.044
	Milk	(kg/d)	32.9	32.6	33.4	32.9	.874
	3.5% FCM	(kg/d)	33.1	32.1	33.0	33.3	.797
	Fat	(kg/d)	1.16	1.11	1.14	1.17	.685
	Fat	(%)	3.59	3.44	3.44	3.57	.414
	Total protein	(kg/d)	1.05	1.06	1.04	1.06	.792
	Total protein	(%)	3.23[a]	3.27[a]	3.14[b]	3.25[a]	.030
	True protein	(kg/d)	1.00	1.02	.99	1.02	.731
	True protein	(%)	3.09[ab]	3.14[a]	3.01[b]	3.13[a]	.022
	Efficiency	(milk/DMI)	1.29	1.29	1.35	1.33	.619
2			39% DM + Urea	39% DM + SBM & MBM	59% DM + Urea	59% DM + SBM & MBM	
	DMI	(kg/d)	24.2[b]	26.2[a]	26.0[a]	26.1a	<.001
	DMI	(%BW)	3.79[b]	4.06[a]	4.09[a]	4.08[a]	<.001
	BW change	(kg/d)	-.25[b]	.57[a]	-.01[b]	.26[ab]	.022
	Milk	(kg/d)	35.4[c]	38.4[a]	35.9[bc]	36.9[b]	<.001
	3.5% FCM	(kg/d)	36.0[bc]	38.2[a]	35.7[c]	37.1[ab]	<.001
	Fat	(kg/d)	1.28[ab]	1.33[a]	1.24[b]	1.30[a]	.024
	Fat	(%)	3.70	3.48	3.47	3.54	.217
	Protein	(kg/d)	1.08[b]	1.15[a]	1.08[b]	1.12[a]	.002
	Protein	(%)	3.08	3.02	3.02	3.07	.452
	Lactose	(kg/d)	1.77[c]	1.94[a]	1.80[bc]	1.85[b]	<.001
	Lactose	(%)	4.98	5.03	4.99	5.00	.662
	Efficiency	(milk/DMI)	1.47[a]	1.47[a]	1.38[b]	1.42[b]	<.001
	Blood glucose	(mg/dl)	62.2	60.0	59.0	60.6	.086
	Blood urea	(mM)	6.57[a]	4.70[b]	6.68[a]	4.65[b]	<.001
	Milk urea	(mM)	6.97[a]	4.79[b]	7.06[a]	4.54[b]	<.001

[abc]($P < .05$ by LSD)

PRODUCTION OF COWS IN EARLY LACTATION FED DIETS CONTAINING ALFALFA OR CORN SILAGE WITH EQUAL RATION NDF CONCENTRATIONS

D.R. MERTENS and A. HALEVI (Volcani Center, Israel)

Introduction

Mertens (1987) developed the NDF-Energy Intake System that maximizes forage use while meeting animal energy requirements. This system is the basis for predicting intake and formulating rations in the animal submodel of DAFOSYM (Dairy Forage Systems Model). When DAFOSYM is used to evaluate the economics of forage systems in a whole-farm setting, it predicts that corn silage results in equal or slightly greater milk production and greater economic return compared to alfalfa. This disagrees with conventional wisdom that dairy cows will consume and produce less when rations contain corn silage. The objectives of this study were to: (1) evaluate the NDF-Energy Intake System, (2) compare alfalfa and corn silage-based feeding systems and (3) identify possible optimal ratios of alfalfa and corn silage for dairy rations.

Materials and Methods

A full lactation feeding trial was conducted with 12 cows per treatment (9 multiparous and 3 primiparous). All cows were fed a diet containing 28% NDF, using 1/2 alfalfa and 1/2 corn silage, for the first three weeks of lactation, and week 3 was used as a covariate period. Only the results of the first eight weeks of lactation are presented in this report. All rations for weeks 4 to 8 of lactation were formulated to contain 27% NDF and 18% CP using high moisture ear corn (HMEC) and soybean meal (SBM) with one of the following forages: AS27 - all alfalfa silage, AC27 - 2/3 alfalfa and 1/3 corn silage, CA27 - 1/3 alfalfa silage and 2/3 corn silage, and CS27 - all corn silage. All rations contained 6% roasted soybeans and were balanced to exceed NRC requirements for Ca, P, K, Mg and S. Corn silage rations were supplemented with sodium bicarbonate.

Body weights and body condition scores were measured weekly. Milk weights were recorded daily and milk composition was determined weekly (WDHIC Appleton Laboratory). Dry matter was determined at both 55°C and 105°C on weekly composites of feed ingredients, total mixed rations and refusals. Feed ingredients were analyzed for CP, NDF and ash biweekly. Rations were reformulated weekly to adjust for changes in DM and biweekly to accommodate changes in CP and NDF of feed ingredients. Neutral detergent fiber was determined using sodium sulfite and amylase. Nitrogen solubility was determined using McDougals's buffer.

Results and Discussion

Both alfalfa and corn silage used in this experiment had similar NDF concentrations (Table 1), and, due to this, the proportion of forage in all rations was similar. However, the rations differed substantially in the ratios of HMEC and SBM due to differences in the CP content of alfalfa and corn silage. Chemical composition of the four rations was similar across all treatments with the exception of nitrogen solubility.

Neither body weights and body condition scores nor their changes differed among treatments during weeks 4 to 8 of lactation (Table 2). Dry matter (DMI) and NDF (NDFI) intakes were not statistically different among treatment averages, but there was a trend toward decreased intake with increasing proportions of corn silage in the ration. Intake of NDF was lower than the optimal NDFI capacity predicted by the NDF-Energy Intake System because rations were formulated to maximize milk production in early lactation and minimize the possibility of fill limiting intake. Based on the actual performance of the cows on each

treatment, the NDF-Energy Intake System would predict that 29 to 30% NDF in the total ration would have maximized performance and forage intake. This is higher than the 27% NDF actually fed. When the lower NDF and higher NEL concentrations of actual rations are considered, the NDF-Energy Intake System predicts an average DMI across all treatments of 3.50% compared to the observed 3.47% of body weight per day.

During weeks 4 to 8 of lactation, milk production and composition did not vary among treatments (Table 3). Recognizing that 25% of the cows were in first lactation, the high level of milk production obtained on all treatments suggests that a wide range of alfalfa and corn silage combinations can be used in early lactation if rations are formulated using NDF. The trend toward lower milk fat percentages with increasing corn silage in the ration suggests that fermentation pattern may be different between alfalfa and corn silage when rations contain 27% NDF.

Conclusions

The NDF-Energy Intake System estimated intakes accurately when the difference between optimal and actual NDF content of the ration was considered. The system predicted that intakes of the cows on this trial were limited by physiological energy demand. There was no difference in milk production among alfalfa or corn silage-based rations during weeks 4 to 8 of lactation, indicating that an optimal combination of these forages did not exist.

Table 1. Chemical composition of feed ingredients and total mixed rations containing various proportions of alfalfa (AS) and corn silage (CS). Treatments were: AS27 = all AS, AC27 = 2/3 AS + 1/3 CS, CA27 = 1/3 AS + 2/3 CS and CS27 = all CS.

Ingredient	Dry Matter	Ash	NDF	CP	Soluble CP	N Solubil.
	(%)			(%DM)		
(%N)						
Feed Ingredients						
Alfalfa silage	41.6	10.9	40.8	21.7	15.5	71.4
Corn silage	34.8	4.2	40.4	7.9	4.5	57.0
HM ear corn	67.0	1.5	14.9	9.4	3.6	38.3
Soybean meal	88.2	6.8	13.5	49.5	15.8	31.9
Roasted soybeans	96.8	5.5	10.9	39.8	4.9	12.3
Rations:						
AS27	53.8	8.3	27.2	18.4	9.8	53.2
AC27	51.8	8.0	27.1	18.1	8.6	47.3
CA27	50.1	8.2	27.0	18.1	7.5	41.7
CS27	48.6	8.4	26.8	18.0	6.4	35.5

Table 2. Body weight (BWT), body condition scores (BCS) and dry matter (DMI) and NDF (NDFI) intakes during weeks 4 to 8 of lactation.

Treatment[a]	Body weight	Weight change	BCS	BCS change	DMI	NDFI
	(kg)	(kg/d)			--(%BWT/d)---	
AS27	582	-0.07	3.58	-0.39	3.50	0.95
AC27	583	-0.17	3.48	-0.25	3.62	0.95
CA27	565	0.08	3.21	-0.36	3.44	0.91
CS27	589	-0.61	3.33	-0.46	3.32	0.86

[a]Treatments described in table 1.

Table 3. Milk production and composition during weeks 4 to 8 of lactation.

Treatment[a]	Milk	4%FCM	Fat	Protein	Lactose	Somatic cells
	-------(kg/d---------		----------------(%)----------------			(1000)
AS27	37.7	35.3	3.58	2.93	4.91	132
AC27	39.0	35.9	3.48	2.90	5.05	90
CA27	40.1	35.4	3.21	2.83	4.86	137
CS27	39.4	35.4	3.33	2.90	4.99	257

[a]Treatments described in table 1.

ALTERED RUMINAL FERMENTATION IN LACTATING COWS FED RATIONS CONTAINING MACERATED ALFALFA

D.R. MERTENS, R.G. KOEGEL and R.J. STRAUB

Introduction

Previous feeding trials indicated that lactating cows in mid to late lactation fed rations containing macerated alfalfa produced similar amounts of milk but gained more body weight and produced milk with slightly lower milk fat percentage. These observations suggest that macerated forages may result in changes in ruminal fermentation that promote lower ratios of acetate:propionate. The objective of this research was to test the hypothesis that macerated forages have different ruminal fermentation characteristics that may be related to differences in chewing activity and ruminal pH.

Materials and Methods

Four lactating cows with ruminal fistula, averaging seven weeks in lactation, were fed total mixed rations containing control or macerated, second-cutting alfalfa harvested as hay or silage. Silages were stored in large round bale bags. Due to limited amounts of forages, the experimental design was a 4X4 Latin square with two week periods. A common concentrate mixture containing 82.1% high moisture corn, 15.0% soybean meal and 2.9% minerals and vitamins was used in all rations and the percentage forage in rations was 60.0, 59.6, 59.8 and 58.9 for control hay, control silage, macerated hay and macerated silage, respec-

tively. Rations were fed at six hour intervals during the day to provide 5 to 15% refusals.

Ruminal samples and chewing data were collected on days 12 and 14 of each period. Ruminal samples were collected from four locations in the rumen every four hours during each day, and the starting time was shifted two hours between days. Ruminal pH was determined immediately after straining samples through cheese cloth using a pH meter that was calibrated at pH 4.0 and 7.0 before every sampling time. A subsample was collected with sulfuric acid as a preservative and HPLC was used to measure volatile fatty acids. Eating, ruminating and drinking activities were recorded every five minutes during each 24 hour period.

Feed intake and milk production were recorded daily and samples were taken twice weekly to determine milk composition (WDHIC Appleton Laboratory). Samples of feeds and refusals were collected daily and composited weekly. Subsamples were dried at 105°C and 55°C for dry matter determination and analyses, respectively. Neutral detergent fiber was determined by the ash-free, amylase modification developed in our laboratory. Chemical composition of forages and rations are given in Table 1.

Results and Discussion

Milk production and composition were not different between treatments although cows produced milk with lower milk fat percentage when fed the macerated forage (Table 2). This agrees with the observations made in the previous trial using lactating cows. Body weight changes were not compared due to the short periods used in this trial. Dry matter and NDF intake were lower for cows fed the rations containing macerated forage, but there were no differences in intake between hays and silages. Lower intakes with similar milk production between treatments suggest that the utilization of macerated forages for milk production was greater than for control forages. Assuming a gain of .12 kg per day (the average of all cows over the trial) and using NRC values for the NEL of high moisture corn and soybean meal, it was calculated that the NEL of macerated and control forages was 1.47 and 1.36 Mcal/kg, respectively. The NEL for macerated alfalfa may be an underestimate because these cows may gain more than controls; however, the increase of 8% in NEL for macerated forages is comparable to the 10% increase observed in the last trial.

Time spent eating, ruminating and in total chewing was less when cows consumed macerated forages, and eating time was less when cows were fed silage-containing rations (Table 3). These results appear to confirm the hypothesis that maceration of forages reduces chewing activity which could cause decreased salivary buffer secretion and altered ruminal fermentation. However, the lack of difference in total chewing activity per kg of NDF suggests that the reduction in chewing activity associated with rations containing macerated alfalfa may be due to reduced intake, rather than changes in chewing requirements when forages are macerated.

Ruminal pH was not different among individual treatments or main effects in agreement with the observation that chewing per unit of NDF was not different (Table 4). The pH's of all treatments were low but they agree with the high concentrations of total VFA's observed for these cows. Our hypothesis that maceration of forages alters ruminal fermentation was confirmed in this experiment. Macerated forages resulted in decreased acetate and increased propionate concentration resulting in a substantial reduction in acetate:propionate ratio.

Conclusions

Maceration of alfalfa alters ruminal fermentation as indicated by increased propionate and decreased acetate concentrations in the rumen. This effect does not appear to be related to changes in chewing activity or ruminal pH. Changes in ruminal fermentation associated with maceration of alfalfa suggest that it would be a beneficial treatment for forages fed to feedlot ruminants.

If altered ruminal fermentation is associated with faster rates of digestion and increased energy utilization, maceration of lower quality (higher fiber) forages may improve their energy availability and intake potential for lactating cows and allow more forage to be included in dairy rations.

Table 1. Chemical composition of forages and total mixed rations.

Treatment	DM	Ash	NDF	CP	NDF	CP
	(%)	(% DM)				
	FORAGES				RATIONS	
Control hay	86.1	10.2	38.7	19.4	28.6	18.2
Control silage	54.8	11.6	38.0	18.9	28.2	18.0
Macerated hay	86.0	9.5	34.6	20.8	26.2	19.1
Macerated silage	49.7	10.4	36.2	20.0	26.9	18.6

Table 2. Body weight (BWT), milk production and milk composition of cows fed control and macerated alfalfa.

Treatment	BWT	Milk	Fat	Protein	Lactose	SCC[a]
	(kg)	(kg/d)	(%)			
Control hay	562.7	35.2	3.11	2.94	5.11	149
Control silage	565.9	35.4	3.29	2.89	5.08	277
Macerated hay	556.6	35.8	2.94	2.95	5.15	422
Macerated silage	555.7	35.7	2.98	2.89	5.30	111
Control average	564.3	35.3	3.20	2.92	5.10	213
Macerated average	556.0	35.7	2.96	2.92	5.23	267
Hay average	559.6	35.5	3.02	2.94	5.13	286
Silage average	560.8	35.5	3.13	2.89	5.19	194

[a]Somatic cell count

Table 3. Dry matter (DMI) and neutral detergent fiber intakes (NDFI) and time spent eating (EAT), ruminating (RUM) and in total chewing activity (TOTAL) per day or per kg of NDF.

Treatment	DMI	NDFI	EAT	RUM	TOTAL	CHEW/NDF
	(kg/d)	(%BW/d)	--------------(min/d)-------------			(min/kg)
Control hay	20.9	1.02	359.4	509.4	868.8	31.2
Control silage	21.1	1.05	279.4	531.3	810.6	28.1
Macerated hay	19.5	0.91	265.6	488.8	754.4	30.7
Macerated silage	20.3	0.97	241.3	479.4	720.6	27.5
Control average	21.0	1.03	319.4	520.3	839.7	29.6
Macerated average	19.9	0.93	253.4	484.1	737.5	29.0
Hay average	20.2	0.96	312.5	499.1	811.6	30.9
Silage average	20.7	1.01	260.3	505.3	765.6	27.8

Table 4. Ruminal characteristics of lactating cows fed rations containing control or macerated alfalfa.

Treatment	pH	Total VFA	Acetic	Propionic	Butyric	Ac:Pr ratio
		-----------------------(mM)-----------------------				
Control hay	5.83	123.3	79.5	26.8	12.4	3.03
Control silage	5.79	123.4	78.4	23.3	16.2	3.49
Macerated hay	5.79	125.3	77.2	31.8	12.4	2.51
Macerated silage	5.82	120.1	70.2	29.7	15.4	2.47
Control average	5.81	123.4	79.0	25.1	14.3	3.26
Macerated average	5.80	122.7	73.7	30.7	13.9	2.49
Hay average	5.81	124.3	78.3	29.3	12.4	2.77
Silage average	5.80	121.7	74.3	26.5	15.8	2.98

RESEARCH PROCEDURES

EVALUATION AND IMPROVEMENTS OF THE SAND-ALUMINA CULTURE TECHNIQUE TO CONTROL PHOSPHORUS SUPPLY

C.J.P. GOURLEY, D.L. ALLAN, M.P. RUSSELLE and P.R. BLOOM

Phosphorus (P) deficiency is a major constraint to crop production on agricultural soils globally. An alternative to the use of P fertilizer is selection and development of plant germplasms that are more tolerant of low P soils. An effective screening technique should: (i) be simple and inexpensive to establish and maintain; (ii) enable large scale evaluation of plant populations; (iii) be reproducible; (iv) permit plants to be grown to physiological maturity; and, most importantly, (v) produce results that correlate strongly with those obtained when plants are grown in the field.

There are few techniques that meet these criteria, but Coltman and colleagues proposed using alumina as a solid phase buffer for P in sand culture. Although this technique has been used to screen for P efficiency of several crop species, there is little information about the use and preparation of the medium or the nature of sorbed P on the alumina surfaces. The objectives of these experiments were to: test a simplified P-loading method, characterize rates of P sorption and desorption from the alumina, test the P supplying capacity of sand-alumina to plants, assess the effect of autoclaving on P supply, and characterize the chemical nature of P sorption by alumina.

Materials and Methods

Alumina, which was activated by heating $Al(OH)_3$, was loaded with P at room temperature by placing 1 kg alumina with 10 L NaCl containing from 0 to 600 $\mu M KH_2PO_4$ in plastic 20-L pails with sealable lids. Five concentrations of P-loading treatments were replicated three times. Containers were manually swirled four times daily for 18 days. Loading solutions were monitored for P concentration ([P]) to assure that steady state conditions had been reached before loading solutions were removed and replaced three times with deionized water. The alumina was air dried before mixing 50-g aliquots with 2950 g acid-washed sand. The mixtures were placed in 3-L, 15-cm diameter pots above a thin sheet of glass wool to facilitate drainage.

Desorption of excess P was achieved by rinsing the sand-alumina with 500 mL deionized water daily for 10 days, followed by similar treatment with a P-free nutrient solution for a further 8 days. Desorption of P was monitored in leachate. After [P] had stabilized, Saranac alfalfa (*Medicago sativa* L.) seeds were sown in six replications of each treatment and seedlings were thinned to four per pot. Nonplanted and planted pots were kept in a greenhouse for 52 days and were watered with P-free nutrient solution about every 5 days. Leachate was collected to monitor solution [P]. To test the effect of autoclaving, subsamples of remaining sand-alumina were autoclaved at 121°C for 2 hours before leaching. Statistical analysis included standard ANOVA and linear regression.

Mineralogical characteristics of nonloaded and loaded alumina were determined using random powder X-ray diffractometry. Changes in surface chemistry were monitored using energy dispersive analysis of secondary X-rays (EDX).

Results and Discussion

These results were obtained by loading alumina with P in plastic containers with sealable lids, demonstrating that complex and expensive mixing equipment, circulating pumps, or process-controlled irrigation systems are not necessary for this method.

Solution [P] achieved in these experiments were similar to other reports, with a range from 2.9 to 88 μ*M*. Final [P] in the sand-alumina medium cannot be precisely predicted, presumably because of variations in P sorption during loading, P release during initial washing, storage time before use, and temperature during sorption and desorption. However, it is generally much less important to have a certain [P] in the medium than to have a sufficient range of [P], particularly at the low end. The following equation can be used to approximate final [P] (μ*M*) in the medium:

$$[P] = 3.01 + (2.40 \times 10^{-4})[P]_l^2$$

where $[P]_l^2$ is [P] (μ*M*) in the loading solution.

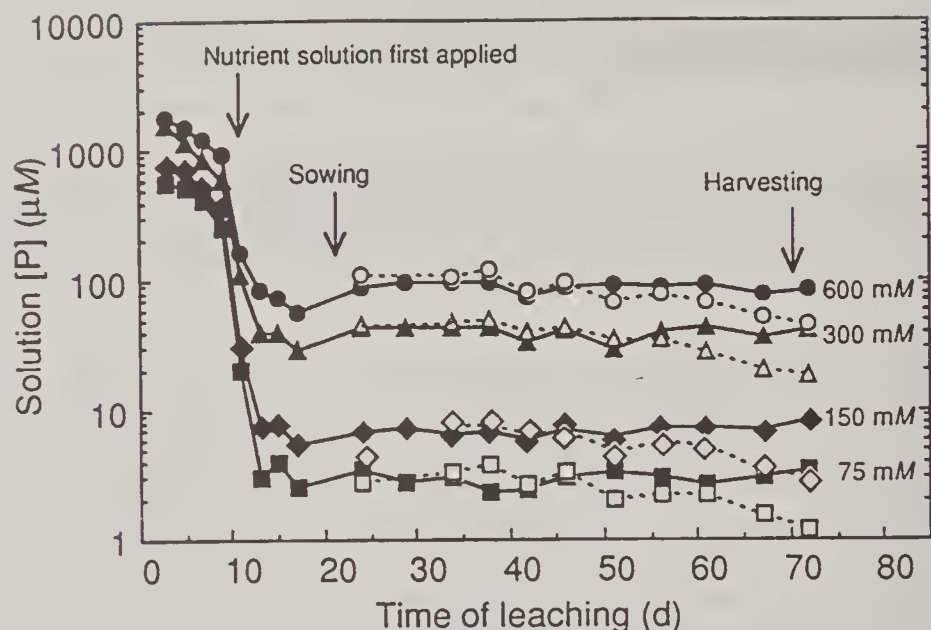


Figure 1. Means and standard deviations of [P] in sand-alumina from four initial P-loading concentrations. Closed symbols were samples collected from pots without plants; open symbols were collected from pots with four alfalfa plants. Standard deviations were smaller than the symbols if not shown.

Phosphorus-loaded alumina provided an almost constant supply of P to the culture medium for 52 days (Fig. 1). With four alfalfa plants per pot, a slow decline in solution [P] was observed, beginning 32 days after sowing, when alfalfa presumably began absorbing P faster than it was released from the alumina. For crops and growth conditions that require longer periods, consistency of P supply may be improved by increasing either the proportion of P-loaded alumina to sand or the volume of medium available for each plant.

Autoclaving the mixed medium resulted in lower [P] than nonautoclaved medium, but supply was constant thereafter (data not shown). We speculate that this change occurred because heat facilitated a more thermodynamically stable arrangement of P-containing compounds, as has been demonstrated in soils. This culture medium could be used in applications where sterile conditions must be achieved.

Structural analysis by X-ray diffractometry indicated that, before P loading, alumina was a crystalline boehmite (AlOOH) and pseudoboehmite, which is a more hydrated form of boehmite having a smaller crystal size. Measurements with EDX on alumina granules loaded with large amounts of P indicated that the surfaces were coated with material with a molar ratio of P:K of about 2.72, indicating the formation of a product with stoichiometry similar to potassium tarakanite. Thus, sorption of P by alumina is actually a complex precipitation reaction rather than a simple adsorption. Variation in [P] is achieved by differences in the ordering of this precipitate and possibly from formation of different products at lower P loading concentrations.

The sand-alumina medium proved to be simple, inexpensive, and effective for growing plants over a wide range of solution [P]. Constancy of P supply was sufficient for most screening experiments. The technique has potential application for the study of plant-microbe interactions, because the medium can be autoclaved.

PROTEIN DISPERSIBILITY INDEX COMBINED WITH 420 nm ABSORBANCE TO EVALUATE THE EXTENT OF HEATING OF SOYBEANS

J.T. HSU and L.D. SATTER

Changes in protein dispersibility index (PDI) in response to different extents of heating have been demonstrated in soybean meal (Circle and Smith; 1980). Eichner and Wolf (1983) observed an increase in 420 nm absorbance due to formation of Maillard products in carrot cubes. The combination of the PDI method and 420 nm absorbance may be useful in distinguishing under-heated, properly heated and over-heated soybeans. The purpose of the present study was to test whether the PDI and 420 nm absorbance could be affected by the following factors: 1) particle size of soybean samples, 2) pH of solvent, 3) temperature of solvent, 4) day to day variation, 5) batch to batch variation and 6) sample to solvent ratio.

Materials and Methods

Soybean samples were heated in a forced-draft oven at 120, 140 and 160°C for 30, 60 and 90 min. Soybeans were ground through a 2 mm screen Wiley mill or 1 mm screen Udy mill for testing the effect of particle size. For testing the remaining variables, only samples ground through a 1 mm screen Udy mill were used. Twenty gram samples of soybeans were blended in 300 ml distilled water at 8,500 rpm for 10 min followed by 939 x 6 rpm centrifugation for 10 min (PDI method-AOCS,1985). The supernatant was analyzed for N content using the Kjeldahl procedure and the PDI value was expressed as % of total crude protein (CP). For the 420 nm absorbance measurement, the same supernatant obtained in the PDI procedure was diluted (80x) and filtered through a Gelman membrane of .2 um pore size before measuring absorbance at 420 nm against distilled water as a blank using a Sargent-Welch spectrophotometer (6-550 UV/VIS). Sodium phosphate buffer (pH 7) was used in contrast to distilled water (pH 6) to test the effect of solvent pH. For testing the effect of solvent temperature, one set of samples was blended and centrifuged in the cold room (4°C) compared to a control set performed at 20°C. For testing day to day variation, the same PDI procedure was performed on the same batch of soybean samples but on a different day. A fixed volume of distilled water (300 ml) was mixed with different amounts of soybean samples (15, 20, 30 and 60 g) for testing the effect of sample to solvent ratio (1:20, 1:15, 1:10 and 1:5). Different batches of soybeans heated in the same way as described previously were used to determine batch to batch variation.

Results and Discussion

The average coefficient of variation (CV) of PDI measurement between the duplicates of all soybean samples tested was 3%. It might be reasonable to consider further replication of analysis if the CV exceeds 5%. There was day to day variation detected in some soybean samples when two months or more elapsed between analysis day, implying some storage effect on the soybeans (Table 1). As expected, PDI values of soybean samples ground through the Wiley mill were lower than those of soybeans ground through the Udy mill (control). Soybean samples processed at 4°C had lower PDI values than those at 20°C (control). The 1:20 sample to solvent ratio resulted in the same PDI estimate as the control (1:15; Table 1). However, other sample to solvent ratios showed some deviation in PDI values. There was no difference in PDI value between solvent pH of 6 and 7. Neither was there a difference between different batches of soybeans heated in the same way. The responses in PDI values and 420 nm

absorbance to different extents of heating are shown in Fig. 1.

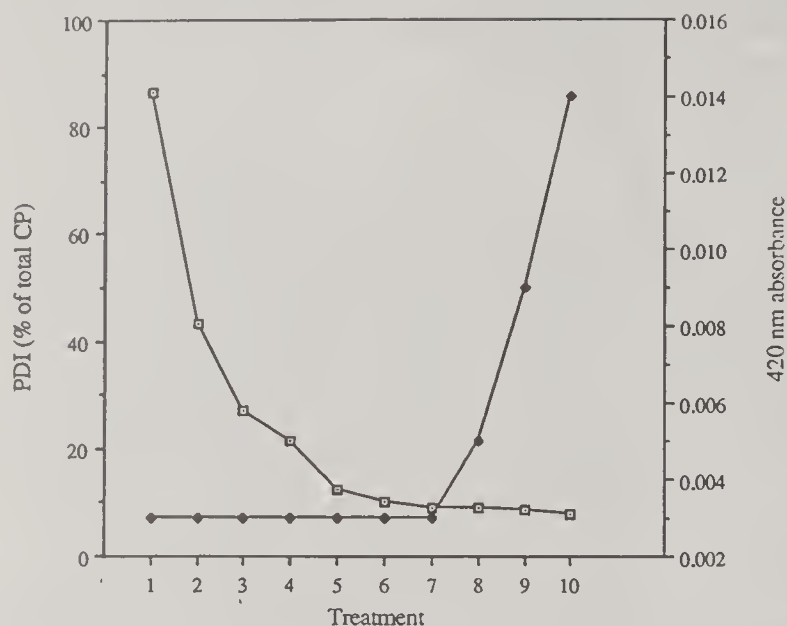


Figure 1. PDI and 420 nm absorbance. Treatments 1 through 10 were raw soybeans and soybeans heated at 120°C 30 min, 120°C 60 min, 120°C 90 min, 140°C 30 min, 140°C 60 min, 140°C 90 min, 160°C 30 min, 160°C 60 min and 160°C 90 min, respectively.

Summary

A fixed sample particle size, sample to solvent ratio and constant temperature should be employed to decrease the variation in the PDI measurement. A combination of PDI and 420 nm absorbance can distinguish between soybean samples heated to different extents.

Table 1. Effects of various factors on the measurement of PDI (% of total CP) of soybeans heated to different temperatures.

	Factor[a]									
Treatment[b]	Control	DD	2 mm	4°C	1:20	1:10	1:5	pH 7	B	SE[c]
1	86.6	87.6	85.4	85.9	86.4	83.6***	83.4***	85.6	87.4	.27
2	43.4	42.8	41.1	37.0	41.2	42.2	53.8	42.2	38.8	2.39
3	27.2	29.1	26.6	25.4	27.3	29.2	31.2	28.4	29.8	.91
4	21.5	21.9	19.9	17.4**	21.2	20.7	23.4	21.6	21.9	.85
5	12.4	12.2	10.1***	11.4**	13.4	13.4	14.1***	13.3	12.2	.23
6	10.3	9.2**	7.6***	8.8***	10.6	10.3	11.2	10.2	9.6	.22
7	9.2	8.6**	7.4***	8.2***	9.4	9.2	9.6	9.2	8.9	.15
8	9.0	8.9	6.8***	8.4	8.9	8.8	10.2	9.8	8.8	.30
9	8.6	7.9	6.5***	7.3***	8.9	8.0	8.9	8.6	8.4	.15
10	8.0	7.6	6.2***	7.3	8.0	7.8	8.9	8.5	8.1	.21

[a]Control (sample ground through 1 mm screen Udy mill, solvent temperature 20°C, 1:15 sample to solvent ratio, distilled water pH 6); DD (day to day variation); 2 mm (sample ground through 2 mm Wiley mill); 4°C (solvent temperature); 1:20, 1:10, 1:5 (sample to solvent ratio); pH 7 (solvent pH); B (batch to batch variation).

[b]Treatments 1 through 10 were raw soybeans and soybeans heated in a forced draft oven at 120°C 30 min, 120°C 60 min, 120°C 90 min, 140°C 30 min, 140°C 60 min, 140°C 90 min, 160°C 30 min, 160°C 60 min and 160°C 90 min, respectively.

**, ***Significantly different than the control at $p < .01$ and .005, respectively.

[c]Standard error for LSD means.

OPTIMAL RANGE OF HEATING OF SOYBEANS AND THE COMBINED PDI AND 420 nm ABSORBANCE PROCEDURE

J.T. HSU and L.D. SATTER

In a previous experiment we found that the combined protein dispersibility index (PDI) and 420 nm absorbance procedure can be used to determine the extent of heat treatment of soybeans. The present study was intended to verify whether animals will respond to differences in the extent of heating soybeans, and whether we can rely on the combined PDI and 420 nm absorbance methods to predict the quality of heated soybeans. The objectives of the present experiment were to 1) verify the optimal range of heating of soybeans by using in situ ruminal protein degradation, blood plasma branched chain amino acid concentrations and milk production changes as criteria, and 2) show how results of the laboratory methods relate to results of animal studies.

Materials and Methods

One batch of raw soybeans (3640 kg) was divided into 12 portions and treated with different roasting conditions (no heating; 110°C-no steep; 110°C-30 min steep; 123°C-no steep; 123°C-30 min steep; 135°C-no steep; 135°C-30 min steep; 146°C-no steep; 146°C-15 min steep; 146°C-30 min steep; 153°C-30 min steep; 160°C-30 min steep) using a Gem-roaster (Winona, MN). All soybeans were tested for PDI and 420 nm absorbance.

Trial 1. Five grams DM of each soybean treatment were used for the in situ study (Stern and Satter, 1984; Nocek, 1985) using two ruminal-cannulated Holstein cows fed ad libitum a diet composed of 78% alfalfa silage, 20% soybeans and 2% vitamin and mineral mix. Samples in dacron bags were inserted into the rumen in reverse order for 0, 1, 2, 4, 8, 16, 32 and 48 h.

Trial 2. Seven of the 12 soybean treatments (raw; 123°C-30 min steep; 135°C-no steep; 135°C-30 min steep; 146°C-no steep; 146°C-30 min steep; 153°C-30 min steep) were used in a feeding study involving 21 Holstein heifers in a 7x7 Latin square design replicated three times. The heifers were fed diets with the same composition as the diet fed in the in situ study, except the soybeans were different. In each period of the Latin square there were 5 d for adjustment and 2 d for blood sampling from the tail vein 5 h after the morning feeding. Plasma amino acids were analyzed using a Beckman Amino Acid Analyzer (Model 6300).

Trial 3. Early lactation cows were fed the same 7 soybean treatments in a Latin square design as in trial 2. Cows were fed diets composed of 50% alfalfa silage, 35.5% high moisture ear corn, 13% soybeans and 1.5% vitamin and mineral mix. Milk samples were collected on the last two days of each period (7 d).

Results and Discussion

PDI, 420 nm absorbance, ruminal protein degradation rate and UIP are reported in Table 1. PDI was able to distinguish between different degrees of heating. The quantity of samples remaining in the dacron bags after 32 and 48 h incubation was not enough for DM and CP analysis in the present study. Therefore, only samples from 0 to 16 h incubation were used to estimate the ruminal CP degradation rate and UIP. Because such a short incubation period was used, the ruminal degradation rates were higher and UIP values were lower than expected. Both in situ CP degradation rate and UIP were linearly correlated ($p < .05$) to PDI ($r^2 = .85$ and .90, respectively). The plasma branched chain amino acid concentrations showed a significant difference among treatments (Table 2). Heifers fed soybeans heated at 146°C-no steep, 146°C-30 min steep and 153°C-30 min steep had higher ($p < .05$) branched chain amino acid concentrations compared to heifers fed raw soybeans. Less extensive heating did not increase concentrations of branched chain amino acids relative to the control. Therefore, they should be considered as under heated.

Cows fed soybeans heated at 146°C-no steep and 146°C-30 min steep showed higher ($p < .05$) 3.5% fat-corrected milk (FCM) production than the control (Table 2). Although feeding soybeans heated at 153°C-30 min steep resulted in the highest plasma branched chain amino acid concentration in the heifer study, it showed a tendency for reduced FCM in the lactation study.

Conclusion

The optimal range of heating is somewhere between 135°C-30 min steep and 153°C-30 min steep. In terms of PDI, 420 nm absorbance, and animal trial results, it appears that 146°C-no steep and 146°C-30 min steep treatments in this study were near optimal. This corresponds to a PDI value ranging between 9 and 11%. While in situ results indicated no further reduction in protein degradation rate with 30 min of steeping, the animal trials suggested some advantage to steeping following roasting at 146°C. We conclude that PDI and 420 nm absorbance can be used to determine if soybeans have been optimally heat treated.

Table 1. Protein dispersibility index (PDI; % of total CP), 420 nm absorbance, in situ protein degradation rate (k_d; h^{-1}) and estimated undegraded intake protein (UIP; % of total CP) of 12 soybean treatments.

Soybeans	PDI	420 nm	k_d	UIP
Raw	86.32[a]	0.004	0.36[a]	7.7[a]
110°C-no steep	67.90[b]	0.004	0.18[b]	17.4[ab]
110°C-30 min steep	53.80[c]	0.004	0.16[bc]	19.2[ab]
123°C-no steep	41.98[d]	0.004	0.16[bc]	18.6[ab]
123°C-30 min steep	30.86[e]	0.004	0.14[bd]	22.5[bc]
135°C-no steep	18.51[f]	0.004	0.10[bde]	27.3[bc]
135°C-30 min steep	14.32[g]	0.004	0.12[bde]	25.3[bc]
146°C-no steep	10.82[h]	0.004	0.07[de]	30.3[bc]
146°C-15 min steep	9.57[h]	0.004	0.08[de]	28.8[bc]
146°C-30 min steep	9.48[h]	0.004	0.09[cde]	29.1[bc]
153°C-30 min steep	9.37[h]	0.006	0.06[de]	34.2[c]
160°C-30 min steep	8.64[h]	0.006	0.06[e]	32.7[c]
SE	0.781	0.001	0.025	4.41

[a,b,c,d,e,f,g,h]Numbers with different superscripts in the same column differ ($P<.05$).

Table 2. Branched chain amino acid concentrations (n mole/ml) in blood plasma of heifers and milk production (3.5% FCM; kg/d) of cows fed soybean treatments.

Soybeans	Branched chain amino acids	Fat corrected milk
Raw	599.5[a]	35.1[a]
123°C-30 min steep	631.4[ab]	35.6[ab]
135°C-no steep	633.5[ab]	35.4[ab]
135°C-30 min steep	612.6[a]	35.9[ab]
146°C-no steep	652.6[bc]	37.8[bc]
146°C-30 min steep	684.8[cd]	38.5[c]
153°C-30 min steep	689.8[d]	35.9[abc]
SE	12.95	0.98

[a,b,c,d]Numbers with different superscripts in the same column differ ($P < .05$).

U.S. DAIRY FORAGE RESEARCH CENTER ANNUAL DAIRY OPERATIONS REPORT, FEBRUARY 1992

L.L. STROZINSKI

The research center herd count stands at 556 (287 cows and 269 herd replacements). We are currently milking 250 cows which are yielding an average of 62 pounds of milk per day. Our DHIA rolling herd average has risen slightly to 18,580 pounds of milk. The reproduction performance of the herd has decreased somewhat during the past year with our average days open climbing to 112 days. Calving interval remains good at 12.6 months but will increase. Hopefully improvements in this area can be made in the near future. The average age of our first calf heifers at calving remains at 24 months and overall I continue to be pleased with the quality, size and performance of our first calf heifers. First calf heifers currently make up 37% of the milking herd and so the average age of our milking herd has moved back to 48 months.

With fiscal year 1991 depressed milk prices, our milk receipts totaled $531,360 while cattle sales brought $81,950. This past fall two of our registered heifers were sold at the University of Wisconsin-Platteville Pioneer Dairy Club sale. I am pleased to report that they brought $4,050 with one animal being the second high in the sale.

Research usage of the herd continues to be high throughout the year with the winter season being the busiest. In fiscal year 1991, 579 animal units were utilized across 16 trials. Presently, 79% of the milking herd is on research trial. This is up from 65% a year ago and is an all time high for our operation. Many animals are assigned to more than one research trial during a given lactation.

We have continued to work cooperatively with various university departments throughout the year. We have provided numerous bull calves for studies on campus. We continue to work with the State Laboratory of Hygiene on a lead project and are now working with the School of Veterinary Medicine on a milk fever project.

Several physical modifications have been made to the livestock facility during the past year. The mechanically ventilated, insulated free stall barn has been converted to a naturally ventilated barn with drop curtain side walls. New energy efficient lighting has been installed. The ventilation system for the milking parlor has also been modified. The observation windows into the milking parlor at the building entrance have been replaced with cement blocks and glazed blocks have been added to the milking parlor walls. A new automatic crowd gate has been installed in the milking parlor to improve efficiency of cow movement through the milking operation. Plans are underway to install tunnel ventilation in our tie stall barns before the summer of 1992. Total modification of the lighting system for the entire facility to a more energy efficient system is planned for 1992.

Emphasis continues to be placed on efficiency of our dairy operation. Plans for 1992 include expansion of the milking herd and restructuring of work assignments to facilitate a reduction in work force. Consideration is also being given to a three times a day milking schedule.

The dairy operation continues to benefit from the efforts of not only an excellent dairy crew but also the cooperation and assistance of the farm crew as well as the maintenance and repair personnel. The performance and extra efforts of all these individuals are greatly appreciated.

U.S. DAIRY FORAGE RESEARCH CENTER
ANNUAL FIELD OPERATIONS REPORT, FEBRUARY 1992

R.P. WALGENBACH

In contrast to 1990, the 1991 cropping season began with above normal and some record breaking warm spring temperatures. Soil moisture levels in early spring were very good, especially compared to those in 1989 and 1990. Early and mid summer precipitation was below normal and this adversely affected crop yields.

The 1991 second and third crops of alfalfa from a lenient 3X cutting schedule (part of a cutting management experiment) produced about half as much dry matter as these crops produced in 1990 (Table 1). A large carry-over of hay from 1990 helped to provide adequate forage supplies for herd requirements in 1991/92.

Soybeans were introduced into our cropping rotation in 1991. About 105 acres were planted and the yield averaged 50.1 bushels/acre. These soybeans were roasted on the farm with a unit that roasted, steeped, roller milled and cooled beans in a continuous flow through system. We will continue to grow about 100 acres of soybeans per year to satisfy herd protein requirements and also to diversify our crop rotations. We also plan to implement no-till seeding of corn into the soybean residue and to investigate other no-till options for wheat, corn and alfalfa seeding. Our long range plans are to eliminate tillage whenever possible.

The 1991 wheat crop produced an average of 46 bushels/acre. In spite of low moisture conditions, the 1991 corn crop produced good yields in most fields. While sparse rainfall occurred, it arrived in a timely fashion. Average yields in the corn variety trial were 173.5 bushels/acre. These yields were produced on a spring plowed former alfalfa field with 200 lbs/acre of 9-23-30 as the only applied fertilizer.

A new 16 x 72 foot bunker silo was constructed in the summer of 1991. A pesticide loading, handling and storage building is currently under construction and it should be completed for the 1992 cropping season. We completed the conversion of a surplus corn dryer from LP to natural gas. We are in the process of evaluating our manure handling system, including everything from the collection pit at the barn to hauling and spreading in the fields.

A new forage handling system which transfers chopped forage onto trucks was used very successfully this past year. Forage is hauled from the field to bunker silos for dumping or to upright silos where forage is dumped onto a platform feeder/blower unit to fill upright silos.

The field operations continue to enjoy excellent effort from the field and maintenance staff as well as cooperation from the barn crew. The center's management and research staff are fortunate to have this group of knowledgeable, cooperative, hardworking and congenial people to work with.

Table 1. Dry matter yields of alfalfa harvested three times over a three year period from three production fields.

Crop	1989	Year 1990	1991
		tons/acre	
First	1.39	1.74	1.71
Second	1.19	1.50	0.86
Third	0.94	0.74	0.34
Total	3.52	3.98	2.91

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